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HPLC-DAD Polyphenolic Profiling and Antioxidant Activities of *Sorghum bicolor* during Germination

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Abstract: The purpose of this study was to assess the suitability of the Romanian Albanus hybrid of *Sorghum bicolor* as a potential functional food ingredient. Ultrasound-assisted extraction in different solvents, together with spectrophotometric and chromatographic methods, was used to monitor the variation in total phenolic and flavonoid content and the antioxidant activity of raw sorghum grains before and during short germination periods (24, 36 and 48 h). The High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) investigation of the extracts revealed that the ungerminated sorghum grains extracted with methanol had the highest diversity of phenolic compounds, while the total phenolic content (TPC) was the highest after 36 h of germination in both extract types: 1853 mg GAE/100 g for the methanolic extract and 1726 mg GAE/100 g for the ethanolic extract. The findings of this study showed that the TPC of sorghum extracts is strongly correlated with their antioxidant activity and, overall, that the studied extracts presented a good radical scavenger activity, which supports the benefits of alimentary uses of *Sorghum bicolor* grains.

Keywords: sorghum grain; germination; phenolic content; flavonoids; antioxidant activity

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1. Introduction

Sorghum bicolor (L.) Moench. is a C4 plant with a high photosynthetic rate characterized by a fast rate of CO_2 fixation, which conducts good productivity in arid climates and even in saline or alkaline conditions [1]. The sorghum plant's wide-range of economical uses such as biofuel and fiber production, animal feed and silage are completed by its increasing importance in human nutrition [2]. A relatively high content of phenolic compounds of some varieties and the possibility of obtaining gluten-free products are some of the advantages of sorghum grains. Other bioactive components of sorghum grains that recommend its use as a functional ingredient are dietary fibers, polyunsaturated fatty acids (PUFAs), phytosterols, policosanols, iron and zinc [3].

In plants, some phenolic compounds are part of the metabolic defense system involved in nonhost resistance, while others have bioactivities such as insectifuge, antifungal, antiviral, UV protection, etc. [4,5]. As they act as signals and protectors, plants may produce them

both during early development and as a response to environmental strains [6]. Thus, the polyphenol content of plants could be augmented by applying pre- or postharvest stress [7,8].

The bioactivity of polyphenols has been linked with their antioxidant activity at a molecular level, such as their free radical scavenging power, metal chelating properties, reduction potential, and also with their ability to activate antioxidant enzymes [9]. Indeed, phenolic compounds target signaling pathways that regulate redox homeostasis, and research shows that they are active agents against neurodegenerative diseases, cancer, metabolic syndrome, and cardiovascular disease [10–12]. However, white sorghum is considered better suited for various food applications, and polyphenol-rich extracts of red sorghums are studied for their pharmacological properties [13]. The neutral aspect and taste of white sorghum flours could gain more consumer acceptance as they are incorporated into non-gluten foods [14].

Sorghum grains, due to the presence of anti-nutritional factors, are usually processed through soaking, fermentation, germination, malting, etc., with the purpose of increasing macronutrient and mineral bioavailability. During processing, the concentration of phenolic compounds could be increased or decreased, depending on the sorghum cultivar, on the processing method and on the method of phenolics extraction [15]. The extraction solvent best suited for extracting total phenolics as well as specific polyphenol classes is variable for each species and plant part [16]. The purpose of this study was to analyze the phenolic profile of sorghum grains and the antioxidant activity before and during germination in order to sustain the beneficial effects of the use of this cereal in food products. Ultrasound-extraction procedures in two different solvents, followed by spectrophotometric and chromatographic methods, were used to monitor the variation in total and specific phenolic content and the antioxidant activity during short germination periods (24, 36 and 48 h).

2. Materials and Methods

2.1. Grain Materials

The *Sorghum bicolor* grains were provided by the S.C.D.A. Secuieni, Com. Secuieni, Neamt, Romania. The hybrid, Albanus (Euralis SAS France), is a white sorghum with a good *Fusarium* sp. and drought tolerance. The cultivation was carried out in a chernozem type soil, with the following characteristics P_2O_5-39 ppm, K_2O-161 ppm, N-index–2.1, pH–6.29, humus content 2.3%. The months corresponding to soil and germination bed preparation were marked by drought. Early vegetation was perturbed by exceptionally low temperatures and heavy rainfall. During the summer months, the drought was severe, with precipitations values of only 122.8 mm. Harvest time was delayed due to heavy rainfall. The production obtained was approximately 10 T grains/ha.

2.2. Seed Germination

Prior to germination, in order to eliminate any potential contamination, the Sorghum bicolor grains were maintained under a UV lamp, at 254 nm, for 10 min. After that, they were submerged in 1% NaClO followed by washing with tap water for 5 min. The germination was allowed at 23 ± 1 °C for 24, 36, 48 h, in darkness, in a germinator (EasyGreen automatic sprouter EGL 55, Biovie Co, Langlade, France). Tap water was sprayed on the grains for 15 min every 4 h. After germination, the germinated grains were dried at 40 °C for 24 h in an oven. All the germinated and raw sorghum grains were milled into powder and used according to the following methods.

2.3. Plant Extractions

For the extraction of Sorghum bicolor compounds, the following samples were used: powdered desinfected and ungerminated grains (SS1), grains germinated for 24 h, powdered (SG 24), grains germinated for 36 h, powdered (SG 36), grains germinated for 48 h, powdered (SG 48).

The extraction procedure was carried out as follows: 20 g of each Sorghum bicolor sample was first subjected to ultrasound extraction with 200 mL of 3:1 hexane/isopropanol in order to remove the lipid content. The delipidized samples were then mixed with 200 mL of alcoholic solvents (methanol or ethanol). The extractions were performed in vessels fitted with an ascending refrigerant, in an ultrasonic bath for 2 h.

The resulting alcoholic extracts were filtered through a filter paper, and then concentrated using a rotavapor until dry [17,18]. The crude obtained extracts were stored at 4 °C until further use.

2.4. Determination of Total Phenolic Content (TPC)

A total of 25 µL of 1 M Folin-Ciocalteu reagent was added to 10 µL of extract. After 5 min of incubation, 25 µL of 20% sodium carbonate solution and 140 µL of ultrapure water were added. Blank samples were prepared by replacing the Folin-Ciocalteu reagent with ultrapure water. After 30 min at room temperature, the absorbance of the samples was recorded at 760 nm using the Tecan Pro 200 multiwell plate reader. Gallic acid and tannic acid (0.97−500 µg/mL) were used as standard references and the results were expressed as equivalents of gallic acid and tannic acid per 100 g sample (mg GAE/100 g and mg TAE/100 g, respectively) [19].

2.5. Determination of Total Flavonoid Content (TFC)

A total of 100 µL of 2% aluminum chloride solution was added to 100 µL of extract, and after 15 min at room temperature, the absorbance of the samples was read at 415 nm using the Tecan Pro 200 multiwell plate reader. The blank samples were prepared with aluminum chloride and ultrapure water. Quercetin and rutin were used as standard references (0.45−250 µg/mL) and the results were expressed as equivalents of quercetin and rutin per 100 g of sample (mg QE/100 g and mg RE/100 g, respectively) [19].

2.6. Antioxidant Activities

2.6.1. 2,2-diphenyl-1-picrylhydrazyl Assay

100 µL of 100 µg/mL 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was added to 100 µL of extract. The absorbance of the samples was measured at 517 nm (Tecan Pro 200 multiwell plate reader) after 20, 35 and 50 min of incubation at room temperature. The blank samples used were the DPPH solution mixed with ultrapure water. Trolox (15.63−250 µg/mL) was used as positive control [19]. The DPPH inhibition percentage was calculated using the following formula:

$$\%\ inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

2.6.2. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Assay

100 µL of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution in methanol (1:60) was mixed with100 µL of extract. The absorbance of the samples was recorded at 734 nm using Tecan Pro 200 multiwell plate reader after 30, 60, and 90 min of incubation at room temperature. For the blank samples, the extract was replaced with ultrapure water [20]. Trolox (15.63–250 µg/mL) was used as positive control. The ABTS inhibition percentage was calculated using the formula from Section 2.6.1.

2.7. HPLC-DAD Polyphenols and Flavonoids Quantification

High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) analysis was carried out using a High-Performance Liquid Chromatography Systems L-3000 from RIGOL TECHNOLOGIES, INC, Beijing, China. The Kinetex EVO C18 (150 × 4.6 mm, particle size of 5 µm) column was used with an injection volume of 10 µL in the chromatographic analysis. The mobile phase consisted of a two-solvent system, used in gradient elution. The solvents used were (A) 0.1% trifluoroacetic acid (TFA) in water

and (B) 0.1% TFA in acetonitrile. The gradient elution was 2−100% B at 30 °C for 60 min and the elution flow was set at 1 mL/min. Five different analytical wavelengths (220, 250, 280, 300 and 320 nm) were monitored in accordance with the literature. Thus, 230 nm was used for chlorogenic acid and genistein, 250 nm was used for rutin, catechin, gallic acid and tannic acid, 280 nm was used for quercetin, 300 nm was used for p-coumaric acid, epicatechin, hyperoside, naringenin and daidzein, and 320 nm was used for caffeic acid.

Identification and quantification analyses were performed by comparison with standards spectra at each retention time. Stock reference solutions at 10, 50, 100, 200 and 400 µg/mL concentration were used for the calibration curves [21]. For each standard reference, R^2 was between 0.998 and 0.999, LOD was ±10 µg/mL and LOQ was equal to or higher than 10 µg/mL.

2.8. Statistical Analysis

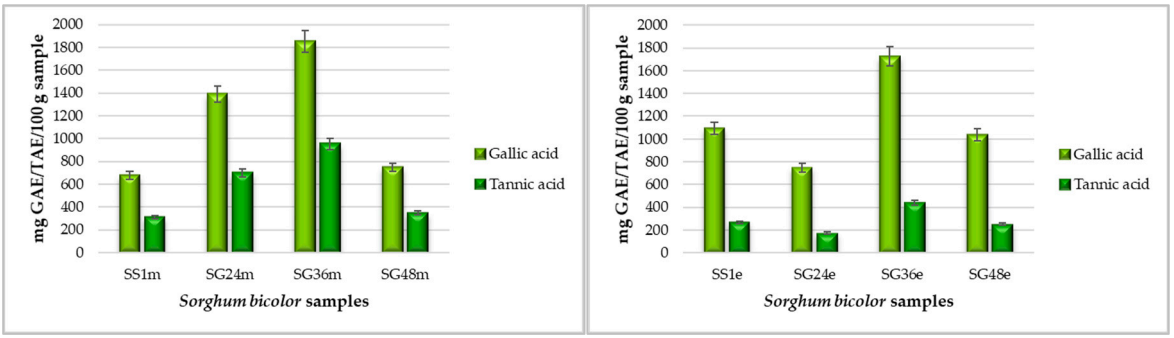
The experiments were performed in triplicate and the obtained data were expressed as the mean ± standard deviation using the Microsoft Excel Program (Redmond, WA, USA) and Origin Software (Bristol, UK). The Student's *t*-Test and Pearson Product-Moment Correlation were performed using XLSTAT (Addinsoft, Paris, France).

3. Results

The aim of our research was to quantify phenolic and flavonoid compounds of a Romanian Sorghum bicolor hybrid, grains and germs in various stages of germination, as well as to evaluate their antioxidant activity. The diversity of the composition in phenolic compounds and flavonoids has been highlighted for methanolic and ethanolic extracts by developing an HPLC-DAD method.

3.1. Total Phenolic Content (TPC)

The total phenolic acids were analyzed from methanolic and ethanolic extracts using a Folin-Ciocalteu micromethod, and the results expressed as gallic and tannic acid are shown in Figure 1.



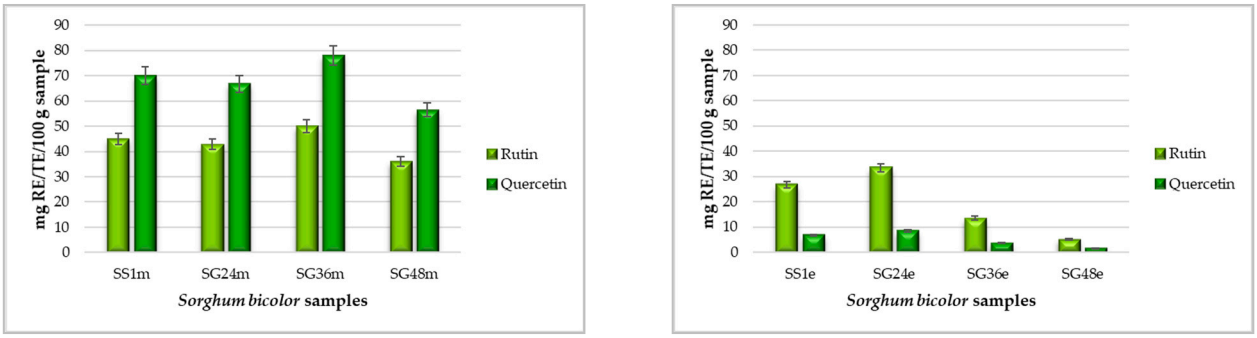
(**a**) (**b**)

Figure 1. The total concentration of phenolic compounds in methanol extracts (**a**) and ethanol extracts (**b**) of sorghum grains.

Both alcoholic extractions resulted in comparable average and total phenolic concentration (TPC). The results obtained with both standard compounds were correlated for each extract type ($r = 1$, $p = 0.01$ (m), $p = 0.02$ (e)). The highest concentration of total phenols was obtained after 36 h of germination: 1726 mg GAE/100 g sample (e) and 1853 mg GAE/100 g sample (m). Gallic acid was found to be the best standard for both extracts. The TPC was similar for the raw sorghum samples and the samples germinated for 48 h. For the methanolic extracts, TPC was 679 and 748 mg GAE/100 g, respectively, while the values for the ethanolic extracts were 1094 and 1038 mg GAE/100 g, respectively.

3.2. Total Flavonoid Content (TFC)

The two solvents were also used for the evaluation of the flavonoid extraction efficiency, expressed as rutin and quercetin. The methanolic extraction procedure proved to be the most efficient for flavonoids (Figure 2a), with the highest amounts being obtained when quercetin was used as a standard reference.



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

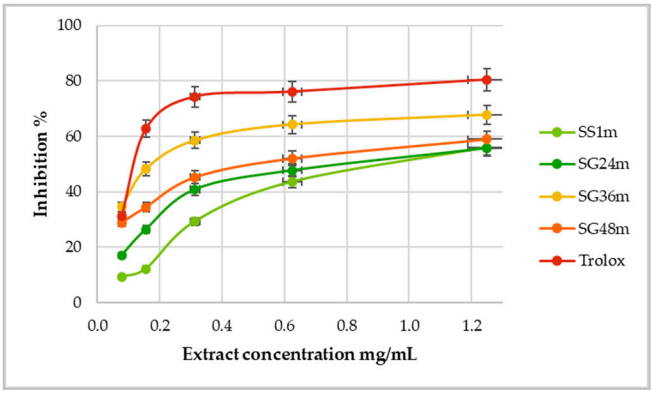
Figure 2. The total concentration of flavonoids of methanolic extracts (**a**) and ethanolic extracts (**b**) of *Sorghum bicolor* samples.

The results obtained with both standard compounds were correlated for each extract type ($r = 0.99$, $p = 0.01$). The highest concentration of flavonoids for the methanolic extracts was obtained after 36 h of germination (78 mg RE/100 g sample), while for the ethanolic extracts it was after 24 h of germination (33.4 mg QE/100 g sample). For both extract types, a decrease in flavonoid concentration was observed after 48 h of germination compared to non-germinated grains, with an 80% loss registered using ethanol extraction.

3.3. Antioxidant Activity Evaluation of Sorghum bicolor Extracts

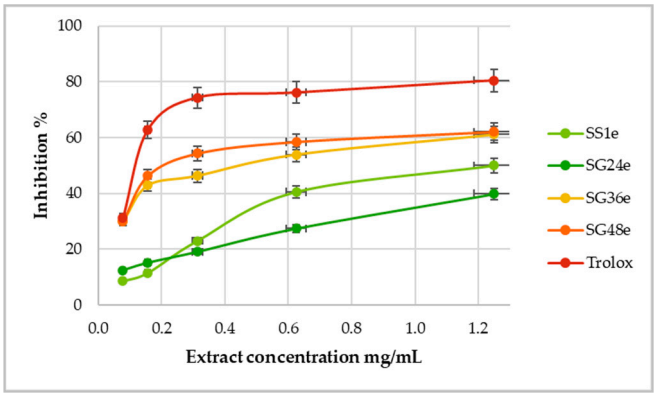
Phenolic and flavonoid compounds from plants have been shown to manifest antioxidant effects. To monitor the relationship between antioxidant compounds from the analyzed extracts and their antioxidant capacity, two different assays were used to scavenge the free radicals (ABTS and DPPH tests).

The studied extracts exhibited good radical scavenging activities both against DPPH (Figure 3) and ABTS (Figure 4).



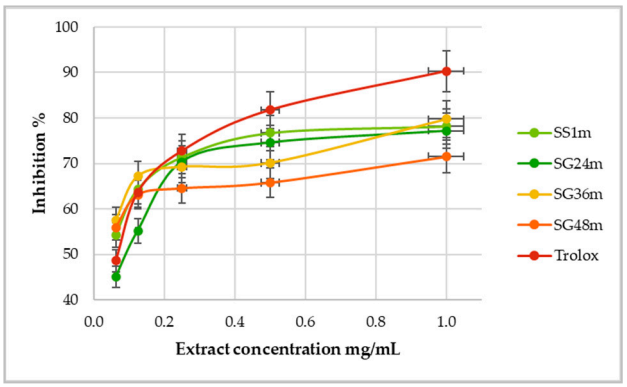
(**a**)

Figure 3. *Cont.*

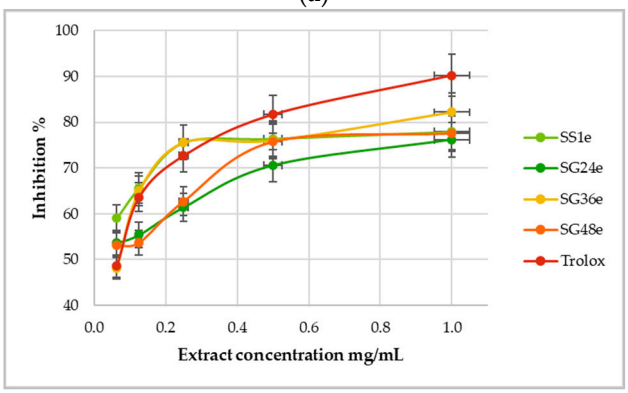


(**b**)

Figure 3. The DPPH inhibition percentage of methanolic extracts (**a**) and ethanolic extracts (**b**) of *Sorghum bicolor* samples.



(**a**)



(**b**)

Figure 4. The ABTS inhibition percentage of methanolic extracts (**a**) and ethanolic extracts (**b**) of *Sorghum bicolor* samples.

At concentrations above 0.1 mg/mL, the DPPH inhibition percentages of the methanolic extracts (Figure 3a) and ethanolic extracts (Figure 3b) are positively correlated with TPC ($r = 0.75$, $p = 0.007$ (m), $r = 0.70$, $p = 0.04$ (e)). The proportion of variance attributed to this correlation is $r^2_m = 0.61$ and $r^2_e = 0.49$. In other words, 61.91% of the DPPH inhibition by the methanolic extracts (49.44% in the case of ethanolic extracts) could be ascribed to the TPC [22].

The inhibition percentage of the methanolic extracts (1 mg/mL) was positively correlated with the total flavonoid content (TFC) ($r = 0.96$, $p = 0.001$), while the ABTS inhibition by ethanol extracts was positively correlated with the TPC ($r = 0.99$, $p = 0.002$). Overall,

the extracts exhibited a higher scavenger activity against ABTS radical cation compared to DPPH radical. The IC_{50} values of the sorghum extracts are presented in Table 1.

Table 1. The IC_{50} values of *Sorghum bicolor* extracts.

Sample Name	IC_{50} (mg/mL)	
	DPPH	ABTS
SS 1m	0.900 ± 0.006	0.027 ± 0.003
SG 24m	0.750 ± 0.005	0.087 ± 0.006
SG 36m	0.300 ± 0.002	0.018 ± 0.001
SG 48m	0.600 ± 0.004	0.022 ± 0.002
SS 1e	1.500 ± 0.010	<0.002
SG 24e	1.900 ± 0.015	<0.002
SG 36e	0.550 ± 0.004	0.063 ± 0.003
SG 48e	0.350 ± 0.003	<0.002

SS 1m/SS 1e—methanolic/ethanolic extract from ungerminated sorghum grains, SG 24m/SG 24e—methanolic/ethanolic extract from 24 h germinated sorghum grains, SG 36m/SG 36e—methanolic/ethanolic extract from 36 h germinated sorghum grains, SG 48m/SG 48e—methanolic/ethanolic extract from 48 h germinated sorghum grains.

In the DPPH assay, the sorghum extract concentrations required for half of the free radicals to be scavenged were generally lower for the methanol extracts. In contrast, the ethanol extraction proved more effective against ABTS, with IC_{50} values lower than 0.002 mg/mL.

3.4. HPLC-DAD Poliphenols and Flavonoids Quantification of Sorghum bicolor Extracts

The major polyphenol and flavonoid compounds with antioxidant activity from our plant matrices were evaluated by HPLC-DAD analysis. The presence of these types of bioactive compounds has been described in several studies related to Sorghum species [23,24]. In this study, the quantification of these secondary metabolites, which have many functions such as antioxidant, anti-inflammatory, antiviral, and anti-carcinogenic activity, was made using an adapted HPLC-DAD method [21].

The chromatographic investigation of the extracts revealed that the ungerminated sorghum grains extracted with methanol (SS1m) had the highest diversity of compounds (Figure 5). The quantification of each studied compound is presented in Table 2.

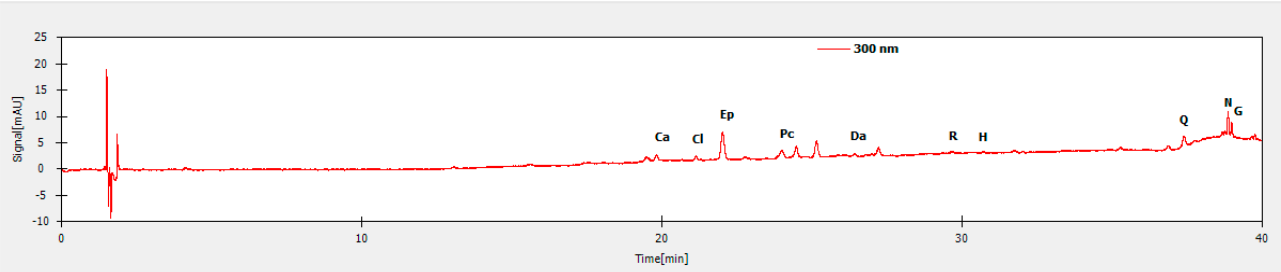


Figure 5. High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) chromatogram of SS1m extract (300 nm). Ca—caffeic acid, Cl—chlorogenicacid, Ep—epicatechin, Pc—p-coumaric acid, Da—daidzein, R—rutin, H—hyperoside, Q—quercetin, N—naringenin, G—genistein.

Table 2. HPLC-DAD quantification of sorghum extracts.

	Concentration (mg/kg)							
Compound	**SS1e**	**SG24e**	**SG36e**	**SG48e**	**SS1m**	**SG24m**	**SG36m**	**SG48m**
Caffeic acid	0.09	0.05	ND	ND	0.25	0.29	0.04	ND
Catechin	ND	ND	ND	ND	ND	0.23	ND	ND
Chlorogenic acid	0.03	0.02	ND	0.05	0.11	0.09	0.04	0.26
Daidzein	0.03	0.01	ND	ND	0.08	0.09	0.03	0.22
Epicatechin	0.85	0.35	0.16	0.58	2.65	3.37	0.55	2.72
Gallic acid	ND	ND	0.05	ND	0.33	0.38	ND	ND
Genistein	0.15	0.08	0.06	0.23	0.40	0.23	0.17	1.15
Hyperoside	ND	ND	ND	ND	0.32	ND	ND	ND
Naringenin	0.12	0.06	0.05	0.19	0.34	ND	0.14	0.96
p-Coumaric acid	0.15	0.08	0.04	0.23	0.41	0.47	0.17	ND
Quercetin	0.06	0.03	0.02	0.06	0.12	0.11	0.05	0.28
Rutin	ND	0.06	ND	ND	0.31	ND	ND	ND
Tannic acid	ND	ND	ND	ND	0.21	0.24	ND	ND

ND—not determined. SS 1m/SS 1e—methanolic/ethanolic extract from ungerminated sorghum grains, SG 24m/SG 24e—methanolic/ethanolic extract from 24 h germinated sorghum grains, SG 36m/SG 36e—methanolic/ethanolic extract from 36 h germinated sorghum grains, SG 48m/SG 48e—methanolic/ethanolic extract from 48 h germinated sorghum grains.

The data presented in Table 1 represent the maximum concentration of each compound across the five monitored wavelengths. In the ethanolic extracts (e), after 48 h of germination, the concentration of chlorogenic acid was almost doubled, and the contents of naringenin and p-coumaric acid were also increased compared to the ungerminated grains. As for the methanolic extracts (m), daidzein and naringenin concentrations were tripled, while the genistein content was increased 7-fold at 48 h of germination. Epicatechin and quercetin were detected in all the tested samples, while tannic acid, catechin and hyperoside were quantifiable only in some of the samples extracted by methanol.

4. Discussion

The climate evolution towards heat and aridity in Romania and the suitability of sorghum for cultivation in semi-arid conditions led to a reconsideration of sorghum as cereal, fodder or a technical plant in the last few years [25]. The aim of this study was to investigate the presence of antioxidant compounds, such as polyphenols and flavonoids, in the grains of a *Sorghum bicolor* hybrid cultivated in Romania, as well as the antioxidant activity, in order to sustain the nutritional value of this cereal and the benefits for its use in human alimentation. The study analyzed the variation in the phenolic and flavonoid profile, as well as in the antioxidant activity during the germination process of sorghum grains, from the perspective of using this cereal in functional foods based on germinated sorghum.

The Albanus white hybrid of *Sorghum bicolor* analyzed in this study was cultivated at S.C.D.A. Secuieni, Com. Secuieni, Neamt, Romania, in unusual climate conditions atypical for the growth and development of field crops. Although the germination bed was dry, the arrival of precipitation after mid-April aided sprouting. Early vegetation was hindered by low temperatures caused by prolonged rainfall. The average precipitation levels in spring in the study area are 139.5 mm, comparable to 137.2 mm registered during the study. A major difference, however, was registered in the rainfall distribution over the months and weeks. The average temperature in spring in the area was 10.6 °C, which was 1.4 °C higher than the multi-annual spring average. The summer months were characterized by extreme drought, afflicting most crops, while sorghum remained resilient. During the study year, summer precipitation values reached only 122.8 mm, with a deficit of 104.7 mm compared to the multi-annual summer average. In addition, temperatures were 1.3 °C higher than the multi-annual summer average. A recent study analyzed the fluctuations of bioactive compounds in sorghum hybrids grown in a temperate climate over a period of three years [26]. The summer weather in Poland was less hot and dry than the studied year in Romania. However, the conclusion of this paper was that the variation in bioactive compounds could not be correlated with the variation in climate conditions, but

rather with the sorghum hybrid type, also suggesting that sorghum is suitable for growth in temperate areas. The authors have determined the TPC after performing ultrasound-assisted extractions using 80% MeOH. Values obtained for the white sorghum variety Sweet Caroline ranged between 822 and 913 mg GAE/100 g, higher than the values obtained in this study using 100% MeOH (679 mg GAE/100 g), but lower than the samples extracted with 100% EtOH (1094 mg GAE/100 g). Another white sorghum hybrid, glasshouse-cultivated in Australia, had a lower TPC of 240 mg GAE/100 g [27]. The extraction solvent used was an acidic mixture of acetone and water.

Methanol was more effective than ethanol for TFC extraction from the samples, which can probably be explained by the highest polarity of the methanol. In the methanol extracts, a great increase in genistein and daidzein concentration was observed after 48 h of germination. During germination, due to increased glucosidase activity, isoflavones become more available as they are released from their conjugates [28]. Other research teams have also analyzed the TFC content of raw white sorghum varieties. For example, the PC 5 hybrid had 15.33 mg RE/100 g, compared to 44.8 mg RE/100 g for SS1m and 26.7 mg RE/100 g for SS1e [24]. However, the cited study used a different assay method. In this study, we have determined that using quercetin as a standard when analyzing the TFC of methanolic extracts yields superior results.

In the HPLC-DAD analysis, low overall values were obtained, possibly due to the prevalence of bound phenolic acids in the tested cultivar [29]. Moreover, low-tannin sorghum varieties such as Albanus have the lowest polyphenol content of the sorghum types [30]. A comparison between the chromatographic results of other published studies and this research work is presented in Table 3.

Table 3. Chromatographic quantification of compounds in white sorghum varieties.

Compound	SS1e (HPLC)	SS1m (HPLC)	Przybylska-Balcerek et al. [23] (UPLC)	Punia et al. [24] (UPLC)
Caffeic acid	0.09 mg/kg	0.25 mg/kg	21.5 mg/kg	14.3 mg/kg
p-Coumaric acid	0.14 mg/kg	0.40 mg/kg	149 mg/kg	8.6 mg/kg
Naringenin	0.12 mg/kg	0.33 mg/kg	1.11 mg/kg	3.6 mg/kg

The findings of our study also show that the TPC of sorghum extracts is strongly correlated with their antioxidant activity and, overall, the radical scavenger activity of the extracts against ABTS radical cation was higher than against DPPH cation, in accordance with the literature [31]. Other studies have also analyzed the TPC and antioxidant activity of raw and germinated sorghum grains. Arouna et al. analyzed a mixture of varieties, extracted with 70% acetone, determining a TPC of 662 and 638 mg GAE/100 g for raw and germinated sorghum, respectively. Furthermore, they observed a significant decrease in DPPH IC_{50} after germination, which was not confirmed by our study [32]. Another publication presents the TPC and biological activities of 50 sorghum varieties before and after germination. While some cultivars have exhibited an increase in the TPC and antioxidant activity after germination, others had a slight decrease. On average, the TPC was determined at 880 mg GAE/100 g before and 920 mg GAE/100 g after germination [33]. In our study, we have also observed only slight modifications of TPC before and after germination. However, the analyzed sorghum hybrid exhibited a significant increase in TPC after 36 h of germination. Our study also revealed that, during germination, changes occur in the phenolic and flavonoid profile, which can positively impact the nutritional value of germinated sorghum grains. To the best of our knowledge, the variation in TPC, TFC and antioxidant activity during germination was analyzed for the first time.

Due to their neutral color and taste, white sorghum cultivars are considered to be beneficial towards the functionalization of sorghum and the acceptability of sorghum foods [14]. Albanus feeding trials conducted on weaning piglets also revealed that this sorghum variety could reduce cholesterol, suggesting a potentially high content of policosanols and/or phytosterols that requires further investigation [34].

5. Conclusions

This study presents the antioxidant activity and phenolic and flavonoid profiles of the Albanus variety of *Sorghum bicolor* cultivated in Romania, before and during germination. A maximum total phenolic content was identified after 36 h of germination. Specific phenolic compounds exhibited diverse variation patterns, and further research is required for the clarification of the biochemical mechanisms involved. Overall, methanol was found to be the more effective solvent for TFC extraction in sorghum grains, while the ethanolic extracts had higher antioxidant activities against ABTS. In spectrophotometric analyses, while the overall trend was not affected in this study, final results could be influenced by the choice of standard references. The variation trend of total phenols and flavonoids throughout the germination intervals was influenced by the extraction method, possibly due to the differences in the extractability of specific compounds. Our findings showed that the Romanian Albanus variety has an average phenolic content which is maintained during germination, even when subjected to stress factors during vegetation and harvest, suggesting its suitability for incorporation into functional foods, especially since this cereal does not contain gluten, thus being suitable in the diet of people suffering from celiac disease. The cholesterol-reducing potential of this cultivar also requires further research.

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