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ORIGINAL ARTICLE / ÖZGÜN MAKALE

BIOLOGICAL ACTIVITY DETERMINATION OF BLACK AND WHITE CHIA SEED EXTRACTS OBTAINED BY DIFFERENT EXTRACTION METHODS

SİYAH VE BEYAZ CHIA TOHUMLARININ FARKLI YÖNTEMLERLE ELDE EDİLEN EKSTRELERİNİN BİYOLOJİK AKTİVİTE TAYİNİ

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ABSTRACT

Objective: *Determination and comparison of the biological activities of black and white chia seeds in extracts obtained by different extraction techniques.*

Material and Method: *Biological activity studies of extracts obtained by different techniques were determined by applying DPPH radical scavenging potential test, TEAC test and ORAC test. In addition, total phenol and flavonoid amounts were determined in methanolic extract samples.*

Result and Discussion: *In this study, phenol amount was determined as 9.86 mg GAE/g for black seed and 12.69 mg GAE/g for white seed and the total amount of flavonoids was determined as 0.098 mg RE/mL for black chia seed and 0.099 RE/mL for white seed. In addition, considering the antioxidant activity tests, the highest antioxidant activity for TEAC test was found in both black and white seed extracts obtained by the Cold press method, the highest activity for DPPH was in the white seed extracted obtained by the Cold press method, and the highest activity for ORAC test was observed in the white seed extract obtained by the Folch technique.*

Keywords: *Biological activity, chia, DPPH, ORAC, TEAC*

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ÖZ

Amaç: *Siyah ve beyaz chia tohumlarının biyolojik aktivitelerinin farklı esktraksiyon teknikleriyle elde edilen ekstrelerde tayinlerinin yapılması ve karşılaştırılması.*

Gereç ve Yöntem: *Farklı tekniklerle elde edilen ekstrelerin biyolojik aktivite çalışmaları, DPPH radikali süpürme potansiyeli testi, TEAC testi ve ORAC testi uygulanarak tayin edilmiştir. Ayrıca metanollü ekstre numunelerinde toplam fenol ve flavonoit miktar tayinleri yapılmıştır.*

Sonuç ve Tartışma: *Bu çalışmada fenol miktarı siyah chia tohumu için 9,86 mg GAE/g, beyaz chia tohumu için ise 12,69 mg GAE/g olarak belirlenmiş olup, toplam flavonoid miktarı ise siyah chia tohumu için 0,098 mg RE/mL ve beyaz chia tohumu için 0,099 mg RE/mL olarak belirlenmiştir. Ayrıca yapılan antioksidan aktivite testleri göz önüne alındığında, TEAC testi için en yüksek antioksidan aktivite, soğuk sıkım yöntemiyle elde edilen hem siyah hem de beyaz tohum ekstresinde, DPPH için en yüksek aktivite, soğuk sıkım tekniğiyle elde edilen beyaz tohum ekstresinde, ORAC testi için en yüksek aktivite ise Folch tekniğiyle elde edilen beyaz tohum ekstresinde görülmüştür.*

Anahtar Kelimeler: *Biyolojik aktivite, chia, DPPH, ORAC, TEAC*

INTRODUCTION

Salvia hispanica L. (Chia) is a species belonging to the Lamiaceae family. Chia; grows naturally in tropical and subtropical environments [1]. It has been cultivated by the Aztecs since pre-Columbian cultures. It has been used as food, either as a whole or in a ground form [2]. Generally, Central and South America are the regions where chia is naturally grown since it has this necessary climate. In addition, it can be grown in the Mediterranean and Southeast Africa [3]. Nowadays chia is considered as a *pseudo-cereal*, which is cultivated for medicinal, pharmaceutical and food usage. Researches around the world have been reporting about the chemical profiles as well as pharmacological and nutraceutical benefits of chia seeds [4].

In literature there are a number of papers dedicated to investigation of many aspects with relevance to agrarian, phytochemical, pharmacological, nutraceutical and medicinal benefits of chia [5,6,7]. Previous investigation on chia seeds resulted with wide range of biological activities: hypoglycemic [8], antimicrobial and antiproliferative [9], hepatoprotective [10], antioxidant and anti-obesity [11]. There were carried out animal studies [12,13] and those that have focused on human participants [14,15] have investigated the seeds' main components by isolating and evaluating them to gain knowledge of their health and nutrition benefits. Research indicates that components of chia seeds are ascribed a beneficial effect on the improvement of the blood lipid profile, through their hypotensive, hypoglycaemic, antimicrobial and immunostimulatory effects. Various chia seeds differed in superoxy dismutase (SOD) activity and exhibited high antiradical activity against 2,2-azino-bis (3-ethylbenzylthiazoline-6-sulfonic acid) (ABTS) [16]. The results show that chia seeds are suitable for use as an antioxidant ingredient in gluten-free diets and health foods of celiac patients [17].

The main results of phytochemical investigations dedicated to chia seeds have concerned with fatty acids composition and phenolic compounds. Kulczynski (2019) has recently gave comprehensive

evaluation on the current state of knowledge about the chemical composition and nutritional value of chia seeds [18]. The fatty acid profile is characterized by abundance of polyunsaturated fatty acids, mainly α-linolenic acid. Linoleic, oleic and palmitic acids are found in lower amounts. Chia seeds have greater contents of omega-3 acids than flaxseed. Chia seeds contain phenolic compounds such as chlorogenic acid, caffeic acid, gallic, cinnamic and ferulic, *p*-coumaric acids, quercetin, rutin, kaempferol, epicatechin, myricetin and apigenin. Isoflavones, such as daidzein, glycitein, genistein and genistin, are found in small amounts [19,20].

Phenolic compounds are widely found in both edible and non-edible plants and have many biological effects, including antioxidant activity. Raw extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are gaining increasing attention in the food industry, as they delay the oxidative degradation of lipids, improving the quality and nutritional value of foods [21].

In this research, a comprehensive scan was carried out, including a comparison of black and white seed unlike previous one. The chia seeds have been subjected to extraction with different extraction techniques to get lipophilic extracts. The extracts of chia seeds were subjected to antioxidants trials against different substrates and through different mechanisms. Thus, it was planned to obtain more detailed and accurate results about the antioxidant activity capacity of chia seeds. The studies on the examining of black and white chia seed extracts biological activities obtained with different extraction methods separately is very limited. The studies on the determination of total phenol and flavonoid amount of chia seeds are similar to those in his study and were carried out by optimizing different methods or using different solvents. In this respect, our study is original and will guide further research.

MATERIAL AND METHOD

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA, 257621), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Sigma Aldrich, Germany, % 97), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma Aldrich, USA, % 98), rutin (Sigma Aldrich, Germany), quercetin (Sigma Aldrich, Germany), gallic acid (Sigma Aldrich, China), potassium persulfate (Sigma Aldrich, Germany), disodium hydrogen phosphate, sodium dihydrogen phosphate, fluorescein (Sigma Aldrich, USA, F6377), 2,2/-Azobis (2-amidinopropan) dihydrochloride (AAPH) (Sigma Aldrich, USA, %97, 440914), sodium carbonate (Merck, Germany), Folin & Ciocalteu reagent (FCR) (Sigma Aldrich, USA, F9252), aluminium chloride (Merck, Germany, 801081).

Plant Material

Black and white chia seeds were purchased commercially from a local market in July 2019 in Eskişehir. In this study, the chia seeds that originated from Argentina were used.

Obtaining Extracts

Black and white chia seeds have been subjected to five different extraction techniques to get lipofilic extracts: accelerated solvent extraction (ASE), continuous solvent extraction (Soxhlet apparate), supercritical fluid extraction (SFE), Folch method and cold pressing.

Antioxidant Activity

Free Radical Scavenging Assay (DPPH Test)

Radical scavenging potentials of the extracts obtained by different extraction methods were tested with DPPH radical [22]. In the experiment, the extract solutions (10 mg/mL in MeOH) and standard substance of gallic acid (0.1 mg/mL in MeOH) were diluted 8 times with methanol using an 8-channel automatic pipettor (Eppendorf Xplorer) to 96-well plate (10; 5; 2.5; 1.25; 0.625; 0.31; 0.16; 0.08 mg/mL) were added. 100 uL sample and 100 uL DPPH (0.08 mg/mL in MeOH) were added into the well and incubated in the dark at room temperature for 30 minutes. At the end of the period, the absorbance values were measured and recorded at a wavelength 517 nm using a spectrophotometer. Methanol was used as a blank and gallic acid solution (0.1 mg/mL) was used as positive control. The results are calculated according to equation (1) over 3x3 repetitions:

$$\% \text{ Inh } = \frac{Absorbance_{\ control} - \text{Absorbance}_{sample}}{Absorbance_{control}} \times 100 \quad (1)$$

Trolox Equivalent Antioxidant Capacity (TEAC) Test

The ABTS radical scavenging effect of extracts obtained by different extraction methods was determined by TEAC test [23]. In order to obtain ABTS cation radical, an aqueous solution of $K_2S_2O_8$ (2.5 mM) and ABTS (7 mM) was prepared. The solution was kept in the dark for 16 hours until the reagent became active. Approximately 3 mL of the prepared stock solution was taken and diluted with absolute ethanol up to the absorbance range of 0.700-0.800 at a wavelength of 734 nm in UV spectrophotometer. The calibration curve was obtained using five different concentrations of trolox (3.0; 2.0; 1.0; 0.5; 0.1 mM in MeOH). In the experiment, 10 uL of the sample solution (10 mg/mL in MeOH) and 990 uL of $ABTS^{\bullet+}$ were placed on a 96-deep well plate and incubated for 30 minutes at room temperature in the dark. Absorbance measurements were made with a spectrophotometer at 734 nm wavelength. By using the calibration curve of trolox, antioxidant capacity values equivalent to trolox were determined [24]. The experiments were performed in triplicate.

Oxygen Radical Absorbance Capacity Assay (ORAC Test)

Oxygen radical scavenging effect of the extracts obtained by different extraction techniques was investigated by ORAC method. It was determined by a method based on the principle of scavenging

peroxyl radicals produced by AAPH [25]. 25 µL of sample solution (10 mg / mL) and 150 µL of 4 nM fluorocein were placed in 96-well plates and incubated for 30 minutes at 37 °C in the dark. Then, 25 µL of 225 mM AAPH solution was added. Then, the mixture was shaken for 10 seconds. Afterwards, the samples were excited at 485 nm wavelength for 180 minutes and left to the plate reader spectrophotometer to emit at 535 nm wavelength and measurements were recorded per minute. Trolox solutions prepared in five different concentrations were used in the study (50; 25; 12.5; 6.25; 0 µM). The results were calculated by calculating the area under the curve equal to the trolox.

Total Phenol Content Determination

Total phenol determination of the extracts obtained from black and white chia seeds was determined by spectrophotometric method using Folin-Ciocalteu reagent (FCR) [25]. For this purpose, 1,0; 0.8; 0.6; 0.4; 0.2 and 0.1 mg / mL of gallic acid solutions in MeOH were prepared and the calibration curve was drawn on the basis of the reaction results with FCR. In the experiment, 20 µL of sample/standard solution, 1.56 mL of distilled water and 100 µL of FCR were added into a 96-well plate. After waiting for about 8 minutes, 250 µL of Na_2CO_3 (20% (v/v) aqueous solution) was added to the wells and incubated for 2 hours at room temperature. At the end of the period, the reaction results were determined by measuring the absorbance values at 760 nm wavelength by spectrophotometer. The results were taken over 3 × 3 repetitions and determined as equivalent to gallic acid [26, 27, 28].

Total Flavonoid Content Determination

Total flavonoid quantification of black and white chia seeds was routinely calculated using $AlCl_3$ reagent as equivalent to quercetin. Rutin and quercetin were prepared at concentrations of 1.0; 0.8; 0.6; 0.4; 0.2 and 0.1 mg / mL. 80 µL of $AlCl_3$ (2 g / 100 mL) reagent and 1.85 mL of absolute ethanol were added onto 80 µL of sample. One drop of acetic acid was used as a blank instead of $AlCl_3$ reagent. After incubation for 40 minutes at room temperature, the absorbance values were recorded at 415 nm. The total amount of flavonoids in the samples were determined by drawing the calibration curve of rutin and quercetin compared with the extracts. The experiments were performed in triplicate [26, 28, 29].

RESULT AND DISCUSSION

In recent years, interest to Chia seeds has tremendously grown due to their high nutritional and medicinal values that approved by number of scientific research works [30].

Antioxidant Activity Results

In order to evaluate the antioxidant potential of the extracts obtained, different antioxidant activity experiments were applied. Antioxidant activity tests are divided into two according to their mechanisms: electron transfer (ET) and hydrogen atom transfer (HAT). Total phenol and flavonoid tests, TEAC assay and DPPH tests were performed by ET mechanism method and ORAC test by HAT mechanism.

DPPH Free Radical Scavenging Results

DPPH radical scavenging effects of black and white chia seed extracts obtained by different extraction methods were calculated as percentage inhibition and are shown in Table 1.

There are few studies on the DPPH radical scavenging for chia seeds in the literature. The DPPH free radical scavenging effect of phenolic compounds in chia seeds was tested and 32.35 µg GAE / mL extract was found [31]. In another the DPPH radical scavenging work of chia, it was determined as 5.63 ± 0.12 mg Trolox / g fresh weight [32]. The reason why the results are different from ours can be the use of different extraction techniques.

Trolox Equivalent Antioxidant Capacity Results

The antioxidant capacity of black and white chia seed extracts obtained by different extraction methods was determined according to the scavenging effect of ABTS radical, and the Trolox equivalent antioxidant capacity values of the samples are shown in Table 1. The calibration curve, equation and R^2 value of the standart antioxidant compound (Trolox) are shown in Figure 1.

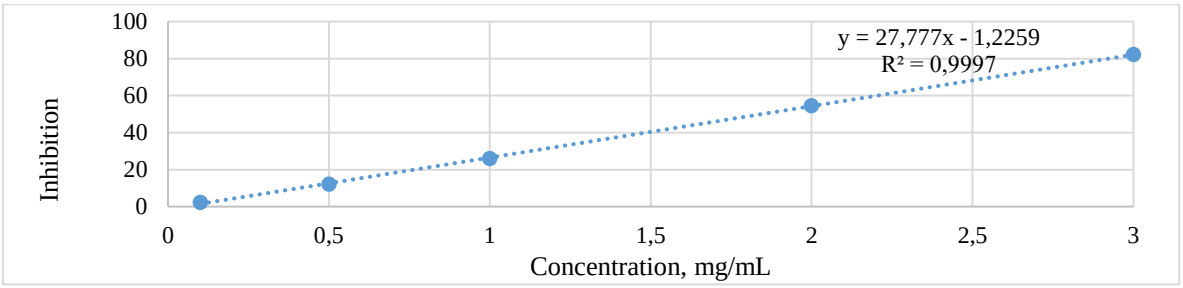

Figure 1. Calibration curve of trolox

As far as we know there is a few TEAC test study for chia seeds. In a previous study, the TEAC test results for chia were found to be 1.317 ± 0.027 and 2.174 ± 0.010 which are lower than our results (approximately between 5.15 ± 0.85 and 10.17 ± 1.14) [33]. In a similar study, the ABTS test results of chia were revealed as 4.73 ± 0.67 mg Trolox / g fresh weight [32]. These values show that the chia seeds we studied have higher antioxidant activity than the previous one.

Oxygen Radical Antioxidant Capacity Results

The oxygen radical absorbance capacity of black and white chia seed extracts obtained by different extraction methods was determined as equivalent to Trolox and the results are given in Table 1. The change of the luminescence values of the fluorescein compound versus time with the effects of chia seed extracts is shown in Figure 2 and Figure 3.

The ORAC test result for chia was found to be 6.48 ± 0.47 µmol TE / g which is lower than our result (ranging between 0.015 ± 0.001 and 0.107 ± 0.002 mM TE / g) in the literature. From these results, we can say that the antioxidant activity of the chia seeds we studied is higher than the other.

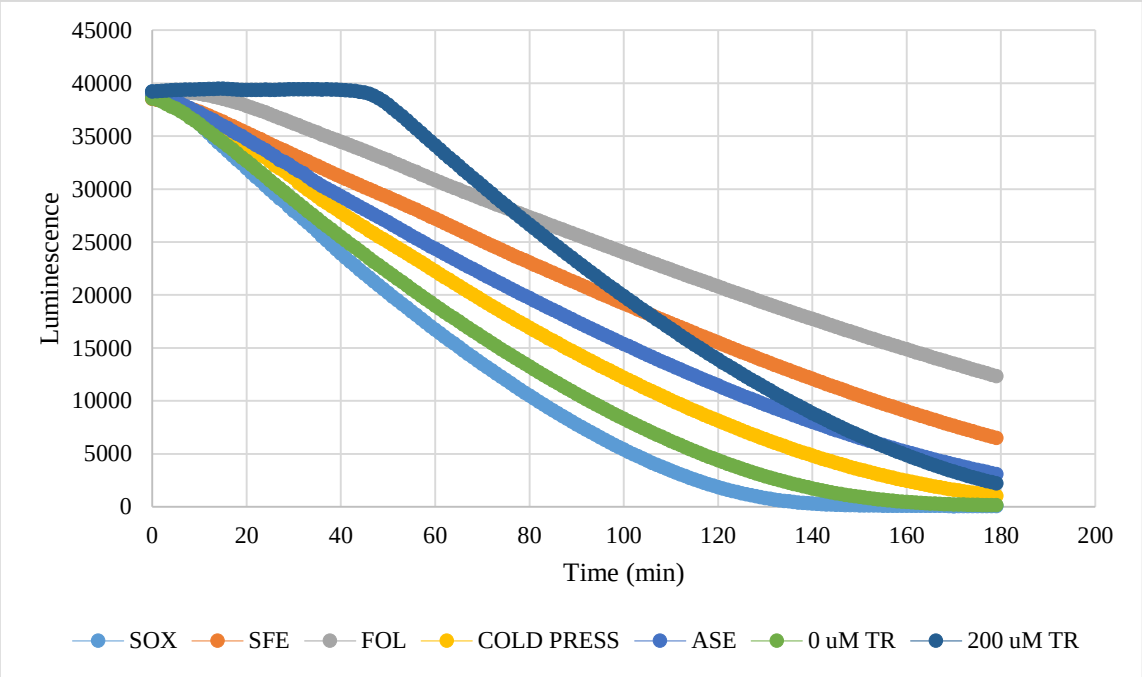

Figure 2. Change of fluorescein luminescence values over time under the influence of black chia seed extracts

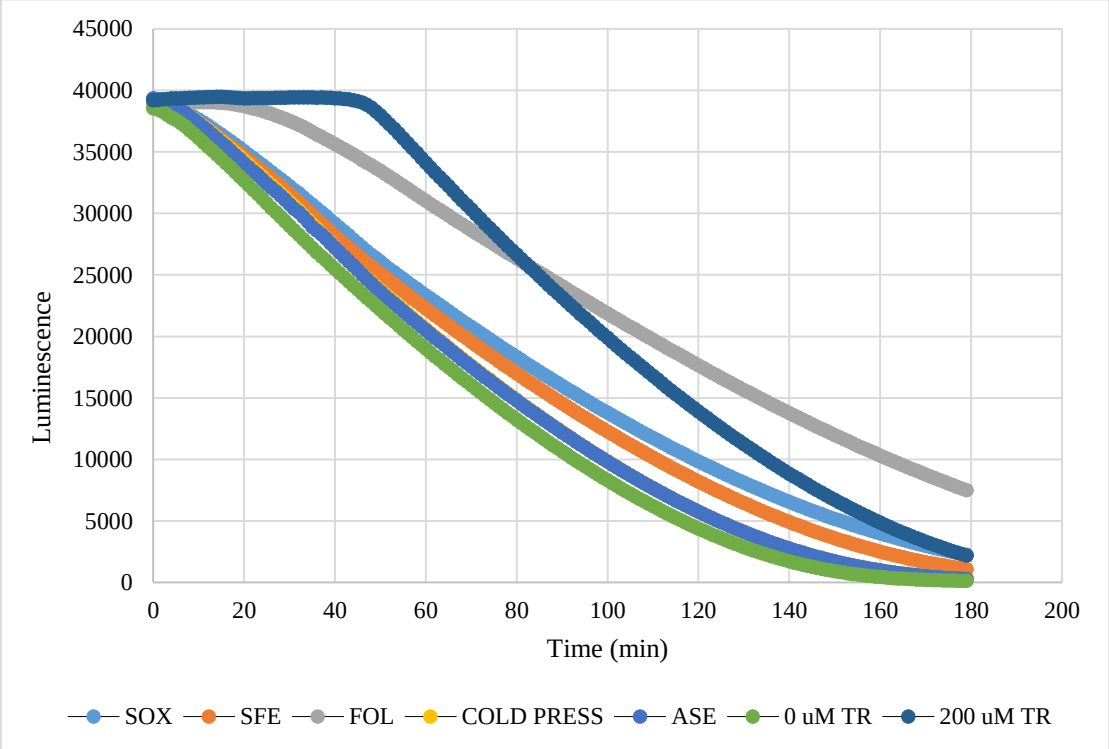

Figure 3. Change of fluorescein luminescence values over time under the influence of white chia seed extracts

Table 1. Antioxidant activities of the extracts obtained from black and white chia seeds with different extraction techniques*

		DPPH** (± SD)	TEAC*** (± SD)	ORAC*** (± SD)
SOXHLET	Black	9.33 ± 1.44	7.22 ± 1.51	0.052 ± 3.437 E - 06
	White	8.61 ± 2.35	7.30 ± 2.13	0.015 ± 9.86 E - 05
FOLCH	Black	19.65 ± 1.95	5.98 ± 0.85	0.056 ± 0.001
	White	19.01 ± 1.68	9.11 ± 1.47	0.107 ± 0.002
SFE	Black	8.24 ± 0.55	7.20 ± 0.82	0.032 ± 0.001
	White	19.66 ± 1.76	6.05 ± 1.19	0.036 ± 0.001
COLD PRESS	Black	15.70 ± 2.59	10.17 ± 1.14	0.049 ± 0.004
	White	20.76 ± 0.80	10.06 ± 0.94	0.015 ± 0.001
ASE	Black	15.41 ± 0.02	7.56 ± 0.64	0.081 ± 0.005
	White	19.08 ± 0.75	5.15 ± 0.85	0.022 ± 0.001

*The concentration of all samples is 10 mg/mL; ** Inhibition %; *** (mM TE/gE), SD: Standard Deviation

Total Phenol Contents Results

Phenol contents of methanolic extracts obtained from black and white chia seeds were determined using FCR reagent. Total phenol amounts were calculated as equivalent to gallic acid and are given in Table 2. The calibration curve of gallic acid (0-1.0 mg/mL) is shown in Figure 4.

Table 2. Total phenol and flavonoid contents of the methanolic chia seed extracts

	Black chia seed (amount ± SD)***	White chia seed (amount ± SD)***
Total Phenol Amounts*	9.86 ± 0.78	12.69 ± 1.73
Total Flavonoid amounts**	0.098 ± 0.002	0.099 ± 0.004

*GAE: Equivalent to Gallic Acid (mg GAE/gextract); **RE: Equivalent to Routine (mg RE/mL); ***SD: Standard Deviation

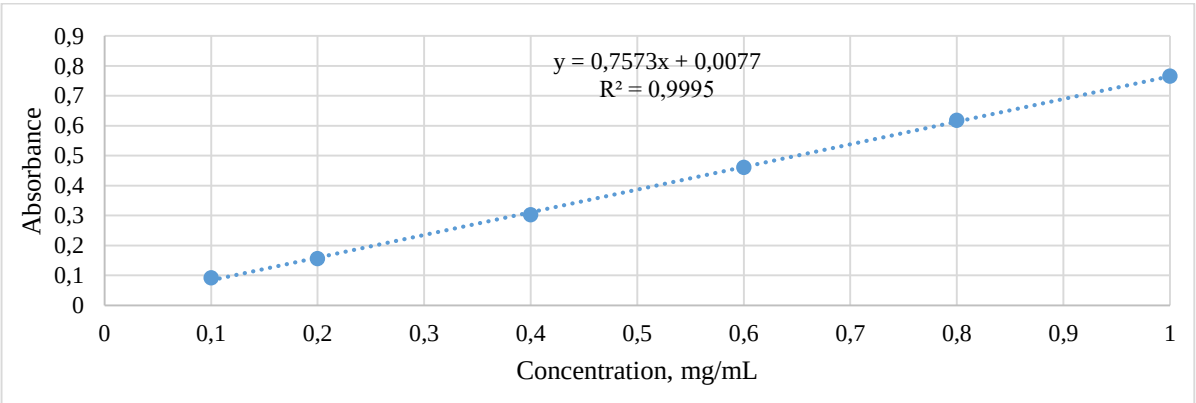

Figure 4. Calibration curve of gallic acid

A previous study in the literature showed that the total phenol content of raw and roasted chia seeds was 3.07±0.01 and 3.43±0.07 mg GAE/g, respectively [34]. The reason for the high phenol content in our study was considered to be due to the extraction procedure performed in the shaker for 24 hours.

Total Flavonoid Contents Results

Flavonoid quantification of the methanolic extracts of black and white chia seeds was determined by $AlCl_3$ reagent, equivalent to rutin. The results are given in Table 2. Calibration curves, equaitons and R^2 values of rutin (0-1.0 mg/mL) shown in Figure 5.

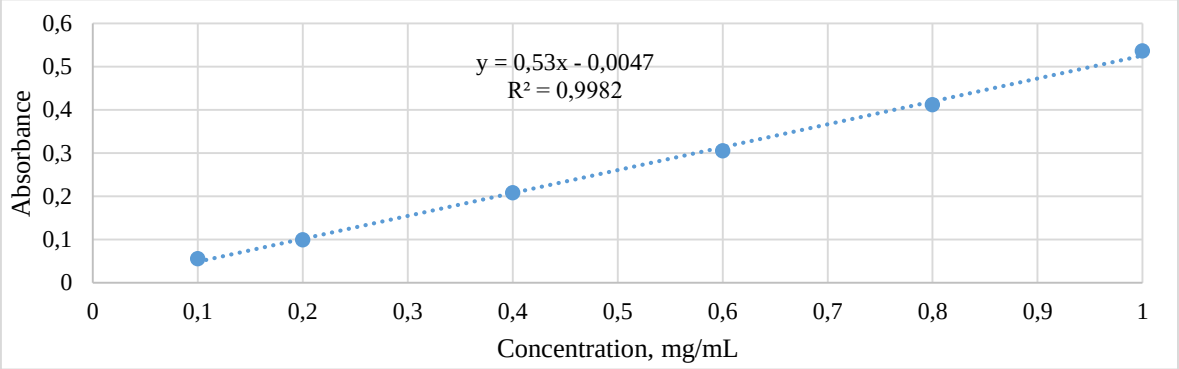

Figure 5. Calibration curve of rutin

In a similar study, the flavonoid content of chia seeds was found to be between 0.055±0.014 and 0.162+0.03 mg QE/g. The reason why the results differ from our study was that different solvents were used and the percentage of solvent was different and it was equivalent to quercetin instead of rutin.

When comparing DPPH radical scavenging effects of black and white chia seed extracts using different extraction techniques, it was observed that the scavenging effect of white chia seed was higher than that of black seed in general. However, the highest activity was determined in the extract obtained by Folch method for black seed. The highest activity in the white seed was determined in the extract obtained by cold pressing technique (Figure 6).

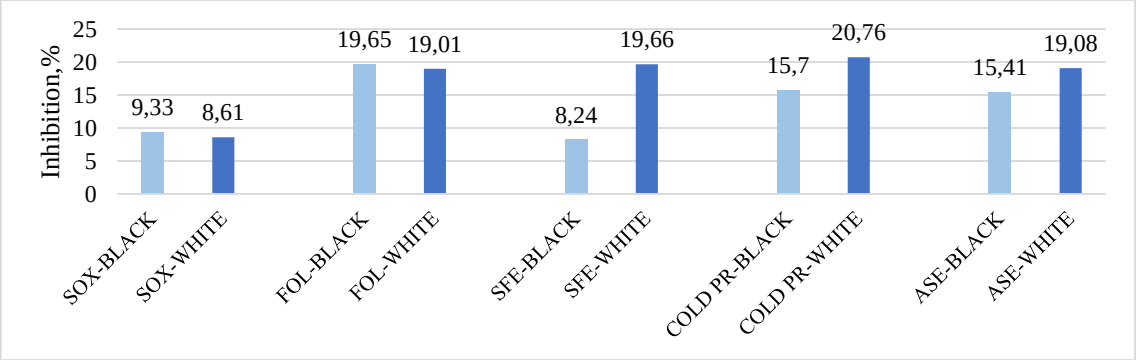

Figure 6. DPPH radical scavenging effect of black and white chia seed extracts.

Considering the scavenging effects of black and white chia seed extracts using different extraction techniques, the highest activity was found in the extracts obtained by cold pressing technique in both seeds. The lowest activity was observed in the extract obtained by the Folch method for black seed, and in the extract obtained by the ASE technique for white seed (Figure 7).

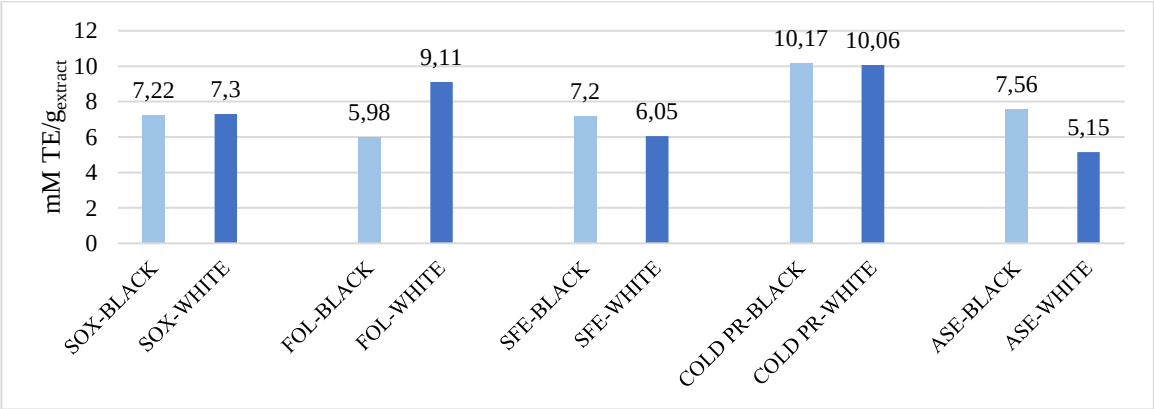

Figure 7. ABTS radical scavenging effect of black and white chia seed extracts

When the scavenging effects of AAPH radical were observed with the ORAC test of black and white chia seed extracts applied with different extraction techniques, the highest activity was detected in the extract obtained by the ASE technique for black seed, while the highest activity for white seed was found in the extract obtained by the Folch method (Figure 8).

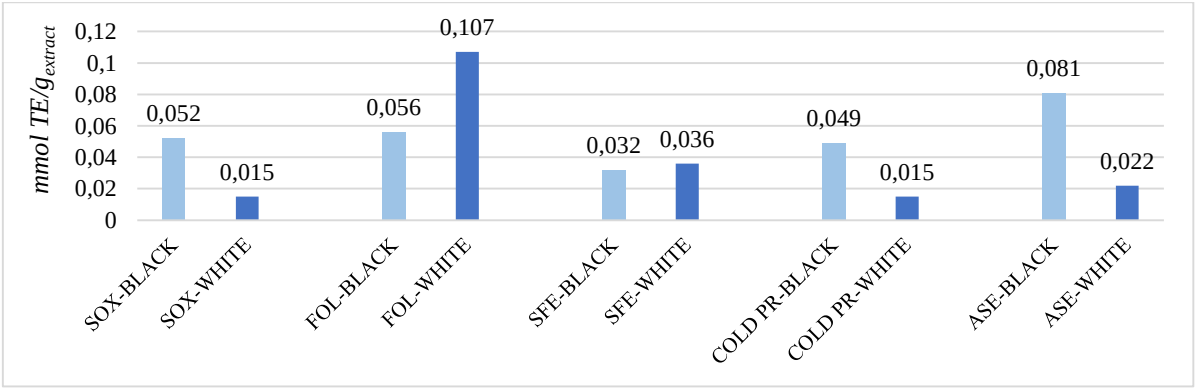

Figure 8. AAPH radical scavenging effect of black and white chia seed extracts by ORAC test

Three different antioxidant activity test results were compared, it was seen that samples obtained by cold pressing technique in DPPH and TEAC experiments had the highest inhibition values. However, in the ORAC test results, it was determined that the extract of the white seed obtained by the Folch method was the most effective. In the both DPPH and TEAC tests based on the electron transfer mechanism, the highest radical scavenging effect was observed for the chia extract obtained by cold

press technique. In the ORAC test based on the hydrogen atom transfer mechanism, the highest radical scavenging effect was observed for the chia extract obtained by Folch method.

In this study, the phenol amount was determined as 9.86 mg GAE/g for black seed and 12.69 mg GAE/g for white seed. The total amount of flavonoids was determined as 0.098 mg RE/mL for black chia seed and 0.099 mg RE/mL for white seed.

Chia is one of the most frequently used as a food for diet programs due to its rich mineral and unsaturated fatty acid content nowadays. This case increases the importance and number of researches on chia.

In conclusion, in this study, the biological activities of chia seeds were investigated using different extraction techniques. The findings, which are different from the previous studies, show that the results of this study will guide the selection of the appropriate extraction technique in the phytochemical analyzes of chia seeds in the future.

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AUTHOR CONTRIBUTIONS

Conception: *T.Ö., G.Ö.*; Design: *T.Ö., G.Ö., Ü.D.U.*; Supervision: *T.Ö., G.Ö., Ü.D.U.*; Resources: *T.Ö., Ü.D.U.*; Materials*: T.Ö., Ü.D.U.*; Data collection and/or processing: *S.Y., Y.D.*; Analysis and/or interpretation: *S.Y., Y.D., G.Ö.*; Literature search: *S.Y., Y.D.*; Writing manuscript: *Y.D., G.Ö., Ü.D.U.*; Critical review: *Ü.D.U., G.Ö.*; Other: -

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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