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# Determination of total phenolic content and antioxidant activities of $M_1$ generation seeds of Chia (*Salvia hispanica* L.) Plant grown in greenhouse conditions

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The purpose of our research was to evaluate phenolic content and antioxidant activity of Salvia hispanica L. seeds grown in greenhouse conditions. The total phenolic content of the extract was determined by the Folin-Ciocalteu method and total antioxidant activity of plant extract was evaluated by standard methods of DPPH and ascorbic acid equivalent antioxidant capacity (AEAC). The purpose of doing this study; To determine the bioactive substance richness in M1 generation chia seed content grown in the greenhouse after invitro study. Total phenolic content has been 31,4241mg GAE/g 100 of gallic acid equivalent and DPPH value 3,38 mg/100 was found in M1 chia seed content grown in greenhouse conditions, Ascorbic acid equivalent antioxidant capacity value 365,6.10-4 g / kg was found.

**Keywords: *Salvia hispanica* L. seed, Total phenolics; Total flavonoids, Antioxidant content, Greenhouse.**

## INTRODUCTION

Plants are good source of biologically active secondary metabolites which have many therapeutic potential in free radical associated disorders (Aydın et al.,,). They are also used in the treatment of some diseases and as foodstuffs. In adition to they are used in the treatment of some diseases due to their medicinal properties. One of the plants used for this purpose is the Chia plant we use in our study. We can give the following information about the general characteristics of this plant in the literature. Although there is a very scientific study about the plant, the difference in the regional and the environments in which it grows also leads to content differences. In spite of the great advances in modern medicine in recent years, plants contribute significantly to health services (Lu et al.,, 2002, Dorman et al.,, 2003, Gu et al.,, 2001, Lu et al.,, 1999, Miliauskas et al.,, 2004,Tepe et al.,, 2006. Chia for commercial purposes has been cultivated in Colombia, Argentina, Peru, Ecuador, Bolivia, Australia and Paraguay. Its seeds are known for their antioxidant properties, dietary fiber and α-linoleic acid contents, which help prevent several diseases (Cahill, 2003, Peiretti et al.,, 2009, Reyes-Caudillo et al.,, 2008, Chicco et al.,, 2009, Bodoira et al.,, 2017, Magdalena et al.,, 2015. Chia seeds contain abundant nutrients. Essential for human health, it is high in protein and rich in elements. It helps to burn more calories, accelerates fat burning Accelerates bowel movements, has the ability to relieve constipation. It protects the heart and brain system and strengthens the immune system and protects against diseases. The purpose of our research is to determine the content analysis of the bioactive substances in the M1 generation product content of chia seeds grown in greenhouse conditions and to determine the difference from the mother.

## MATERIALS AND METHODS

### Plant Material

Chia (*Salvia hispanica* L.) seeds of Bolivian origin, obtained from Genartek Biotechnology Company were grown in greenhouse condition. Chia seeds were sown in March and completed the plant development process in ten months. M1 seed generation was obtained from greenhouse grown plants. Seeds, basal medium (MS) 3% sucrose, 7% agar with 0,4 mg l-1 gibberellic acid ($GA_3$) and 10 mg l-1 were grown in vitro in the medium supplemented with ascorbic acid (AA) (Murashige et al.,, 1962). Seeds were collected in November-December after completing their development. Chia seed germination was to within one week. Chia seedling completed its development process in about eleven months (Figure 1).


**Figure 1. Chia plant and seeds grown in greenhouse condition.**

### Extracts preparation of seed

The seeds collected were shredded with a blender in our laboratory. Twenty-five gram of each sample was transferred to Erlenmeyer flasks and 250 ml of ethanol (96% Merck) was then added as solvent. The extraction was carried out by standing in a temperature-controlled water bath apparatus for 6 hours. The extract was then separated from the sample residue by filtration through Whatman No. 1 filter paper. The solvent portion of the extracts obtained was removed in a rotary evaporator (IKA, RV 10 basic V-C, Germany). All of the extracts were lyophilized and stored in the refrigerator at + 4 ° C. until use. After sample (2 g) was taken from chia seeds, 10 ml of 96 % ethanol was added and mixed for 2 minutes in the homogenizer. It was kept in a water bath at 45°C for 1 night and at the end of this period, it was centrifuged at 4000 rpm for 5 minutes. The supernatant part was taken and blown in a rotary evaporator until it was completely dry at 45°C. The extracts were dissolved in 1 ml of ethanol for phenolic analysis and made ready for analysis (Kiselev et al., 2007). The rol-B gene–induced over production of resveratrol in Vitis amurensis transformed cells. The total phenolic content of extracts was determined using to the Folin-Ciocalteu method. Preparation of saturated sodium carbonate: For 100 ml of solution, add 20 g of sodium carbonate to 80 ml of pure water and boil well. It is cooled down to room temperature. Then another 7 g is added. It is kept in the dark for 24 hours after nucleation. Then it is filtered through filter paper. The volume of the bottom part is completed to 100 ml with distilled water. 40 microliters of extract was added to the tubes from Chia seed extracts by putting 2.4 ml of pure water. With 200 microliters of Folin-Ciocalteu, 600 µl of saturated sodium carbonate was brought to room temperature with the condition of being between 30 seconds and 7.5 minutes, and 760 µl of pure water was added and vortexed. It is waited for 2 hours in the dark at room temperature and a reading is made at 765 nm on a spectrophotometer

### Total antioxidant capacities

The antioxidant capacity of the extracts was evaluated using the ascorbic acid Equivalent Antioxidant Capacity (AEAC).The stock solution was prepared as follows: (0.2mg) in 1 ml of chloroform was added to 20µL of linoleic acid, and 200 mg of Tween -20 emulsifier mixture. The mixture was then evaporated at 40 ºC for 10 min by means of a rotary evaporator to remove chloroform. For control, 0.2 mL of solvent ethanol was placed in test tubes instead of the extract. As soon as the emulsion was added into the testtubes, initial absorbance was measured with a spectrophotometer (UV- Shimadzu, Japan) to be at 470 nm. All samples were assyed in triplicate. The antioxidant activity was calculated using the equation below Zaspel et al., 1983. Calculation: Sample evaluation was performed qualitatively first and then quantitatively with the external standard - calibration curve method (Singleton et al., 1965). The radical scavenging activitywas calculated in terms of Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) by using the following formula:

AEAC= A control – A sample / A control–A ascorbic acid ×

concentration of ascorbic acid × (mg/mL) × volume × 100/g of sample

Where, A is Absorbance (at 517 nm).

(A0; for example, initial absorbance, at initial absorbance of control, A00; absorbance of the

sample after 120 minutes, At 0; absorbance of control after 120 minutes).

**Free radical scavenging activity (DPPH)**

Free radical scavenging activity of ethanol extracts of *S. hispanica* was measured by 2 2'-diphenyl-2-picrylhydrazyl (DPPH; Sigma Aldich, Steinheim, Germany) using 0.1 mM solution of DPPH in ethanol was prepared. Then, 1 mL of this solution was incubated with varying concentrations of *S. hispanica* seeds (1-500 µg/ mL). The reaction mixtures were then shaken well and incubated for 30 minute in dark at room temperature. The free scaveging capacities of *S. hispanica* M1 seeds ethanolic extracts of seeds was evaluated and determined as follows;

AA: [1-(A0-At/A00-At0 )] x 100

**RESULTS AND DISCUSSION**

Plants containing high phenolic compounds can be a good source of antioxidants and total phenolic contents (Kara 2017). In our study, Chia (*Salvia hispanica* L.) seeds with rich nutrient content were grown by germinating under different environmental conditions and new generation seeds were obtained. The extractions of the new generation seeds obtained were analyzed and the phenolic, antioxidant content was tested. The grown M1 generation seeds content and germination rate and duration were evaluated in terms of the physiological characteristics and development of the plant.

**Total phenolic and flavonoid content**

Total phenolic and flavonoid contents in the ethanol extracts from *S.hispanica* M1 seeds were measured in this research. The results are as follows; chiagenic acid 25,11 µg/10 g, caffeic acid 54,22 µg/10 g and quersetin 1,713 µg/10 g and it was revealed that different environmental conditions affect the content of phenolic substance. In this research total phenolic content of Chia seeds M1 generation obtained by growing under greenhouse conditions was determined. It has been determined that the total phenolic content of Chia seeds grown in greenhouse conditions is high and total phenolic amount 31, 4241 mg GAE /g in seeds extracts. The results exhibit that the seed extract of *S. hispanica* evaluated, the ethanol extract, showed stronger antioxidant activity. Antioxidant activities of ethanolic extracts of the *S. hispanica* were determined via 2 2-diphenyl-2-picrylhydrazyl radical scavering assay. DPPH is one of the antioxidant analysis methods. Total antioxidant value (3,38 µmol TE/g) found in chia seeds grown in greenhouse conditions was found to be high. Results showed that high antioxidant and free scavering capacities. It has been determined that Chia seeds $365{,}6.10^{-4}$ g/kg obtained from plants grown in greenhouse conditions. The presence of chlorogenic acid, caffeic acid and quercetin, which are among the phenolic compounds, has been determined in chia seeds. According to a study; the phenolic and antioxidant content of *S.hispanica* seed was examined. It has been determined that the chia seed content of Chia with the oil content removed, the white chia seed phenolic content is higher than the normal seed and there is a significant difference in DPPH free radical sweeping activities. Previous studies have shown that chia seed composition is significantly affected by geographical location (Yeşiloğlu et al., 2013). For instance, chia grown in Peru was found to contain higher in total phenolic contents of chia seeds harvested form different locations were also observed. The total phenolic activity of Chilean chia seed has been reported to be 0.94 mg gallic acid equivalent of sample (Tunçil et al.,2019). Quercetin content was found to be higher in Chia seeds grown in land conditions compared to chia seeds grown in greenhouse conditions. Different environmental conditions have greatly affected the total amount of phenolic substances contained in Chia seed content. It has been determined that the amount of antioxidant substance in chia seed content varies according to the ambient conditions in which the plant is grown (Zaspel et al., 1983, Silva et al., 2014). In our study, it was determined that M1 chia seeds extract grown in greenhouse conditions have more antioxidant content than seeds grown in land conditions. It has been determined that different environmental conditions greatly affect the amount of ascorbic acid contained in chia seeds (Tunçil et al.,2019).

Our results demonstrated that all extracts of *S. hispanica* have efficient compounds in grown greenhouse. The M1 generation of the chia plant seed grown in the greenhouse is very rich in extract content. In terms of both phenolic content and antioxidant content, this was also determined by our analysis. In conclusion, M1 chia seeds showed high concentrations of vitamin, total phenolic and antioxidant content. The reason for the differences in its chemical composition of the seeds grown in the greenhouse is due to different grow location, soil conditions, temperature, humidity, light and growing conditions.

**CONFLICT OF INTEREST**

The authors declared that present study was

performed in absence of any conflict of interest.

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**AUTHOR CONTRIBUTIONS**

Yeşim Kara: Conceived the idea, planned for the study and writing the article.My experimental work was done by my high degree student Nesrin Erim.



**REFERENCES**

Aydın Ç, Taşdelen G, Turan M, Mammadov R. 2016. Phenolic contents and antioxidant properties of Eichinops *ritro* and E. *tournefortii* Jaup. Et. Spach extract. Int. J. Sec. Met., 3(2): 74-81.

Bodoira RM, Penci M C, Ribotta PD, Martínez ML. 2017. Chia (*Salvia hispanica* L.) oil stability: Study of the effect of natural antioxidants. Food Sci. Technol. 75: 107-113.

Cahill JP. 2003. Ethnobotany of Chia *Salvia hispanica* L. (Lamiaceae). Economic Botany., 57*:* 604-618.

Chicco AG, D'Alessandro M.E, Hein GJ, Oliva ME, Lombardo YB. 2009. Dietary chia seed (*Salvia hispanica* L.) rich in α-linolenic acid improves adiposity and normal-ises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. British J. nutrition. 101(1): 41-50.

Dorman HJD, Peltoketo A, Hiltunen R & Tikkanen MJ. 2003. Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chemistry. 83: 255-262.

Gu L, Weng X. 2001. Antioxidant activity and components of Salvia plebeia R.Br.-a Chinese herb. Food Chemistry., 73: 299-305.

Kara, Y. 2017. Phenolic contents and antioxidant activity of jojoba (*Simmondsia chinensis* L.) Schindler. International J. Second. Met., 4(2):142-147.

Kiselev KV, Dubrovina AS, Veselova MV, Bulgakov VP, Fedoreyev SA, Zhuravlev YN. 2007. The rol-B gene–induced over production of resveratrol in *Vitis amurensis* transformed cells. J. Biotechnol., 128: 681-692.

Lu Y, Foo LY & Wong H. 1999. Sagecoumarin, a novel caffeic acid trimer from *Salvia officinalis.* Phytochemistry. 52: 1149-1152.

Lu Y, Yeap F. 2002. Polyphenolics of *Salvia*-a review. Phytochemistry., 59: 117-140.

Magdalena PA, Quesada I, Nadal Á. 2015. Prenatal exposure to BPA and Off spring Outcomes: Dose Response. 13(2): 48-55.

Miliauskas G, Venskutonis PR & Van Beek TA. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry., 85: 231-237.

Murashige T & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant., 15: 473-97.

Murashige T & Skoog FA. 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant., 15: 473-97,

Peiretti P &Francesco G. 2009. Fatty acid and nutritive quality of chia (*Salvia hispanica* L.) seeds and plant during growth. Animal Feed Sci.Technol., 148(2): 267-275.

Reyes-Caudillo E, Tecante A, Valdivia-López, MA. 2008. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. Food Chem., 107: 656-663.

Silva RM, Marinelia E, Moraesa S, Alves l, Adriana T, Godoyb M, Noguire E, Mario RMJ. 2014. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.) LWT - Food Science and Technology. 59(2*):*1304-1310

Singleton VL, Rossi JR. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphhotungstic acid. Am J. EnolViticul., 16: 144-158.

Tepe B, Sökmen M, Akpulat AH & Sokmen A. 2006. Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry., 95: 200-204.

Tunçil YE, Çelik ÖF. 2019. Total phenolic contents, antioxidant and antibacterial activities of chia seeds (*Salvia hispanica* L.) having different coat color. Akademik Ziraat Dergisi., 8(1): 113-120.

Yeşiloğlu Y, Sıt L, Kılıç I. 2013. Invitro antioxidant activity and total phenolic content of various extracts of *Satureja hortensis* L. collected from Turkey. A. J. Chem. 25(15): 8311-8316.

Zaspel BJ, Csallany S. 1983. Detemination of alpha-tocopherol in tissues and plasma by high-performance liquid chromatography. Anal Biochem., 130: 146-150.