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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SPANISH SAGE *(SALVIA HISPANICA L.)* INTRODUCED IN THE RUSSIAN FEDERATION

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Abstract. *Chia (Salvia hispanica L.) is an annual herbaceous plant belongs to the Lamiaceae family. Considering the consumer's interest in healthy and nutritious foods, the cultivation of chia crop has been globally extended. The current study consists in the creation of introduced varieties of chia in the Russian Federation and the interrelation of environmental factors of southern forest-steppe of Western Siberia. This crop has been cultivated in Mexico since ancient times, while in Russia it has not been grown yet. Until the present, numerous previous studies have focused on the study of a single morphological part of chia plant and especially on the seeds, sometimes on leaves and never on stems. The objective of this study was to determine the total phenolic content (TPC) and its antioxidant activity of chia Seeds, Stems and Leaves (chia SSL). The total phenolic content was measured by the FolinCiocalteau method with some modification to adapt the assay to 96-well microplates, using gallic acid as standard while the antioxidant capacity was based on the ability of these plant extracts (chia SSL) to scavenge DPPH radical. 70% TPC methanol extraction and 70 % TPC ethanol extraction were compared, with exception of chia seeds extracts significant differences were found in chai leaves and chia stems. High total phenolic content was detected in Chia leaves (9.183 ± 0.0625 mg GAE/g methanol extract and 7.809 ± 0.157 mg GAE/g ethanol extract), more than 3.2-fold higher than previously reported, followed by chia Stems (7.819 ± 0.225 mg GAE/g methanol extract and 6.695 ± 0.626mg GAE/g ethanol extract) for the first time determined, and chia Seeds (1.669 ± 0.079 mg GAE/g methanol extract and 1.614 ± 0.040 mg GAE/g ethanol extract) higher than previous report. Chai Leaves (92.24%) exhibited the strongest antioxidant capacity followed by chia Stems (74.43%) and chia Seeds (41.35%). Indeed, 70% ethanol and 70% methanol extracts showed similar DPPH scavenging activity. It can be concluded that the surveyed parts of the Russian-Siberia grown chia plants could be potential sources of high phenolic content and antioxidant agents.*

Keywords: *antioxidant activity; chia, Salvia hispanica L., solvent extraction, total phenolic content.*

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Оригинальная статья

ОБЩЕЕ СОДЕРЖАНИЕ ФЕНОЛОВ И АНТИОКСИДАНТНАЯ АКТИВНОСТЬ ШАЛФЕЯ ИСПАНСКОГО (SALVIA HISPANICA L.), ИНТРОДУЦИРОВАННОГО В РОССИЙСКОЙ ФЕДЕРАЦИИ

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Аннотация. *Чиа (Salvia hispanica L.) – однолетнее травянистое растение, относящееся к семейству ламиевых. Учитывая интерес потребителей к здоровым и питательным продуктам, выращивание культуры чиа было расширено во всем мире. Текущее исследование заключается в создании интродуцированных сортов чиа в Российской Федерации и взаимосвязи факторов окружающей среды южной лесостепи Западной Сибири. Эта культура культивируется в Мексике с древних времен, в то время как в России ее еще не выращивали. До настоящего времени многочисленные предыдущие исследования были сосредоточены на изучении одной морфологической части растения чиа и особенно на семенах, иногда на листьях и никогда на стеблях. Целью данного исследования было определить общее содержание фенолов (TPC) и их антиоксидантную активность в семенах, стеблях и листьях чиа (chia SSL). Общее содержание фенолов измеряли методом Фолина Чокальто с некоторой модификацией для адаптации анализа к 96-луночным микропланшетам, используя галловую кислоту в качестве стандарта, в то время как антиоксидантная способность была основана на способности этих растительных экстрактов (chia SSL) поглощать радикал DPPH. Сравнивали 70%-ную экстракцию метанолом и 70%-ную экстракцию этанолом, за исключением экстрактов семян чиа, существенные различия были обнаружены в листьях чая и стеблях чиа. Высокое общее содержание фенолов было обнаружено в листьях чиа (9,183 ± 0,0625 мг GAE/г экстракта метанола и 7,809 ± 0,157 мг GAE/г экстракта этанола), что более чем в 3,2 раза выше, чем сообщалось ранее, за которыми следуют стебли чиа (7,819 ± 0,225 мг GAE/г экстракта метанола и 6,695 ± 0,626 мг GAE/г этанольного экстракта) впервые определено, а семена чиа (1,669 ± 0,079 мг GAE/г метанольного экстракта и 1,614 ± 0,040 мг GAE/г этанольного экстракта) выше, чем в предыдущем отчете. Листья чая (92,24 %) обладали наибольшей антиоксидантной активностью, за ними следовали стебли чиа (74,43 %) и семена чиа (41,35 %). Действительно, экстракты 70 % этанола и 70 % метанола показали сходную активность по удалению DPPH. Можно сделать вывод, что исследованные части растений чиа, выращенных в России и Сибири, могут быть потенциальными источниками высокого содержания фенолов и антиоксидантов.*

Ключевые слова: *антиоксидантная активность; чиа, Salviahispanica L., экстракция растворителем, общее содержание фенолов.*

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1. Introduction

Spanish Sage (Salvia hispanica L.) commonly known as chia, is an annual plant of Lamiaceae family. It is cultivated as a native plant crop in Southern Mexico and Northern Guatemala [1]. In 2009, chia seeds were approved as a novel food by the European Union, and can be used up to 5% in bread making [1]. As a consequence, industrial production of chia seeds and food products containing chia seeds has been rapidly growing in many parts around the world including Mexico, Argentina, Australia, Bolivia, Colombia, Ecuador, Nicaragua and Paraguay [2]. Chia seeds contain a high amount of dietary fiber superior quality protein, omega-3 fatty acids, vitamins, minerals and wide range of phenolic antioxidants [3], [4], [5]. It contains a high content of oil (25–32%), protein (18.5–22.3%) and fiber (20.1–36.15%), as well as 59.9–63.2% of Alpha-linolenic acid (ALA) and 18.9–20.1% of linoleic acid [3]. Furthermore, Scapim et al., [6] and Kobus-cisowska et al., [7] stated that the chemical composition of each product can vary due to different factors such as year of cultivation, environment of cultivation, and extraction method used. Majority of the species from genus salvia have horticultural and homeopathic character as a basis of numerous biological active substances, like phenolic compounds, such as caffeic acids and chlorogenic as well as flavonoids, namely myricetin, quercetin and kaempferol [8].

Phenolic compounds are extensively distributed in plants and serve as important components in our daily diets. As secondary metabolites, phenolics are synthesized in plants from phenylalanine and to a lesser extent tyrosine during normal growth and development as well as in response to stress conditions [9] [10]. In plants, phenolics have multiple functions from general fitness regulation to defense mechanisms against insects, pathogens, UV light and extreme environmental conditions [9]. Phenolics are also responsible for the bitterness and astringency, colour, odour, and oxidative stability of foods [10]. As dietary bioactive, phenolics and polyphenolics exhibit various functional and biological activities but these actions depend on their chemical structures [11], [12].

Up to now more than 8000 phenolic compounds have been recognized in seeds, fruits, vegetables, and related products. Most common phenolics are simple phenols, phenolic acids and their derivatives, flavonoids and their derivatives, coumarins, stilbenes, lignans and their polymerized counterparts like tannins and lignins [10]. Phenolics and polyphenolics are known as powerful antioxidants that inhibit oxidative deterioration of foods and may protect human body against oxidative stress-mediated diseases. Phenolics are excellent free radical scavengers, metal chelators, singlet oxygen quenchers, reducing agents and synergists with other antioxidants. They prevent oxidation of biomolecules such as LDL-cholesterol, membrane lipids, proteins, and DNA and diminish related disorders such as inflammation, carcinogenesis and atherosclerosis [13]. Due to high diversity of secondary metabolites like phenolic compounds, chia seeds possess excellent antioxidant capacity as well as antimicrobial activity and are also used against several pathological disorders, cardiovascular diseases, diabetes, antiviral, antifungal, anticancerogenic, cancer and anti-inflammatory properties [14].

Unlike chia seed research, a few studies deal with chia plant leaves composition and antimicrobial properties [15], [16]. Elshafie et al., [16] reported that the principal constituents of essential oil extracted from aerial parts of this plant were sesquiterpenes with a mainstream of caryophyllenes.Furthermore, the authors determined that this product could be potentially used for microbial control regarding the antimicrobial effect.

The massive nutritional and therapeutic potential of chia plant is little known, chia plant offers a great future perspective for feed, food, medical, pharmaceutical and nutraceutical sectors [17]. The present study aimed to explore the total phenolic contents of introduced samples of Spanish sage (*Salvia hispanica L.)* in Omsk State Agrarian University named after P.A. Stolypin, Omsk, Russia, through the Folin-Ciocalteu (FC) method, of chia plant seeds, leaves and stems extracts, obtained using good polarity solvents, and to compare methanol and ethanol extraction. Moreover, this study also aimed to identify the antioxidant capacity (AC) of chia plant seeds, leaves and stems extracts of by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and further use of the extracts in the food industry.

2. Materials and methods

2.1. Chemicals and Reagents

Methanol (99.99%), ethanol (99%), Folin–Ciocalteu (FC), Sodium carbonate, DPPH (1,1-Diphenyl-2-picrylhydrazine), gallic acid, all obtained from Chem Express (Russia). Deionized water, were used for dilutions for all measurements and all chemicals used in this study were of analytical or food grade.

2.2. Cultivation, Sampling and Drying of Chia Plants

The plant, Chia seeds, stems and leaves (Chia SSL) used in this study were cultured at the experimental field belonging to the Omsk

State Agrarian University named after P.A. Stolypin, Omsk, Russia. The culture was done in the conditions of Western Siberia, it blooms in July-August, the seeds ripen in September-October by triplicate. The samples were taken to the laboratory of the department of Agronomy within the same day, and the green mass and the seeds of chia were manually separated from extraneous matter, labeled, and dried at 35ºC in the stove until constant weight. Then after, chia SSL samples were ground in the laboratory of International Research Center “Biotechnologies of the Third Millennium”, ITMO National Research University, Saint Petersburg, Russia using mill A11 basic BS32 IKA Mill, São Paulo, for 10 s and repeated 5-6 times. The chai SSL powders were passed through a sieve mesh 40 (0.420 mm). The samples were stored in sealed bags at -20 ºC in airtight bags until being used.

2.3. Preparation of TPC extracts

The TPC were extracted using the methods proposed by Martínez-Cruz and Paredes-López [18] with some modifications. A mass of 0.5 g of chia seeds, leaves and stems were extracted with 3 mL of aqueous methanol (methanol: water, 70:30 v/v), (ethanol: water, 70:30 v/v) for 24 h at 25ºC under mechanical shaking, in triplicate. The mixture was centrifuged at 6,000 rpm for 10 min, and the supernatant was stored at −20ºC until further analysis.

2.4. Total phenolic content

TPC was determined by the Folin-Ciocalteu assay following previously reported protocols Martínez-Cruz and Paredes-López [18] with some modifications to adapt the assay to 96-well microplates. Briefly, 237 μL of water and 3 μL of the crude extract were mixed with 15 μL of water-diluted Folin-Ciocalteau reagent (1:1, v/v). Then, 45 μL of sodium carbonate (7.5% w/v) were added and the mixture was incubated for 2 h at ambient temperature (25ºC). The absorbance was measured at 760 nm in a Benchmark plus microplate spectrophotometer reader (Bio-rad, Hercules, CA) and compared with a previously prepared gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram sample (mg GAE/g of Chia SSL). A calibration curve ranging from 20 to 200 μg ml−1 was used to quantify the TPC content in the seed extracts. All the measurements were performed in triplicate for each sample analyzed.

2.5. DPPH Radical Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed as described by Corral-Aguayo [19]. Briefly, aliquots of 280μL of 100μM DPPH/methanol solution and 20μL of crude extracts were added to a 96-well plate. Twenty microliters of methanol were placed in the first row as a blank. The plates were incubated for 30min in the dark and absorbance was read at 517nm ina Benchmark plus microplate spectrophotometer reader (Bio-rad), the measurements were performed in triplicate. Antioxidant activity was expressed as an inhibition percent of DPPH radical and calculated from the equation:

(%) Inhibition = [(Abs control – Abs sample)] / (Abs control)] x 100

3. Results and discussion

Chia seeds are considered a good source of different components, particularly due tomainly the crop, and environmental and agronomic factors. In this work, a phytochemicalanalysis of the components of chia seed, chia stems and Chia leaves fromRussian Federation grown in Western Siberia was carried out,with the aim of determiningits phenolic composition, antioxidant activity and its potential benefits for human health. Our results describe that the main componentsand functional activities are biological active substances like phenolic component, reinforcing the concept of chia as a complete andfunctional food.Studies on the chemical components of plant products are always a challenge, as a huge number of different families of compounds can be existing, in different concentrations and in different forms, specifically in a free form or linked to other constituents from the cell walls. The lack of data concerning the chemical composition of chia plant products is recognized. As the usage of chia plant products is growing, it is significant to undergoes research studies to determine the biological active substances of chia plant seeds, stems and leaves. In an attempt to support in the analysis of the total phenolic constituents existing in the crude extracts, they were subjected to 70% ethanol and 70% methanol solvents, and technology. The extraction process can be used for different purposes, depending on when it is applied. In our study the 70% aqueous methanol extract, 70%aqueous ethanol extract, mechanical shaking, centrifuge and spectrophotometer were applied to determine the total phenolic content and to analyze their antioxidant capacity. The total phenolic content (GAE/g) expressed as gallic acid equivalents of methanol extract sample mg/g of chia grown in Russian-Western Siberia, Chia SSL extracts and antioxidant activity are expressed in percentages. Values are the mean ± SD of three determinations.

3.1. Total phenolic contents

Results obtained for total phenolic content (TPC) ofSpanish Sageseeds extracted by 70% methanol extract and 70% ethanol extract were 1.669±0.079 mg GAE/g and 1.614±0.040 mg GAE/g respectively.

The total polyphenolic content of Spanish Sageseeds found in this study was higher compared to the values reported by Porras-Loaiza et al., [20], which evaluated the phenolic content of Spanish Sage seeds from different locations in Mexico and reported an average of 0.9, respectively 0.66 mg GAE/g. The result is in accordance with Martínez-Cruz and Paredes- López [18] 1.64 mg GAE/g sample. Moreover, this result is comparatively higher than the data already reported in literature by Falco et al. 0.50 [21], Amato et al. 0.530 mg GAE/g chia seeds [22]. However, the value obtained by 70% methanol and 70% ethanol extraction were lower compared to the data reported by da Silva Marineli et al., [23], Saphier et al., [23]: 1.99 mg GAE/g sample, and Yi Ding et al., [24]: 2.39 mg GAE/g sample. Furthermore, this value is higher than the data reported for mango (0.560 mg GAE/g), papaya (0.576 mg GAE/g), and pineapple (0.0258 mg GAE/g) [25]. As chia plant is consumed in parallel with cereals or replacing them in the human diet it is interesting to compare the results obtained in our study with the content reported for cereals. The TPC in the crude extracts of Spanish Sage seeds were higher than those reported by Irakli et al., [26], for extracts of barley (Hordeum vulgare, 0.37 mg GAE/g), oat (Avena sativa, 0.39 mg GAE/g), rice (Oryza sativa, 0.24 mg GAE/g) and corn (Zea mays, 0.15 mg GAE/g).

Total phenolic content of Spanish Sage plantleaves were 9.183 ± 0.0625 mg GAE/g methanol extract and 7.809 ± 0.157 mg GAE/g ethanol extract. Generally, TPC of the green mass of Spanish Sage plantis rarely studied. TPC of Spanish sage plant leaves is more than 3.2-fold higher than reported in literature by Wong et al., [27] 2.43 mg GAE/g *Schismatoglottisahmadii* (Leaf) ethanolic extract. However, it is lower than reported by J. Y. Wong et al., 10.61 ± 3.12 *Aniseiamartinicense* (Leaf) ethanolic extract [27].

Total phenolic content ofSpanish Sage stems in methanol extracts was 7.819 ± 0.225 mgGAE/g and in ethanol extracts was 6.695 ± 0.626mg GAE/g. As we have explored for the literature, it is for the first time determined from chia stem plant. This result is more than 2-fold than edible plant extracts like *Heckeriaumbellatum* (Stem) methanolic extract 2.88 mg ± 0.59 GAE/g and 2.06 ± 0.28 ethanolic extract reported by Wong et al., [27].

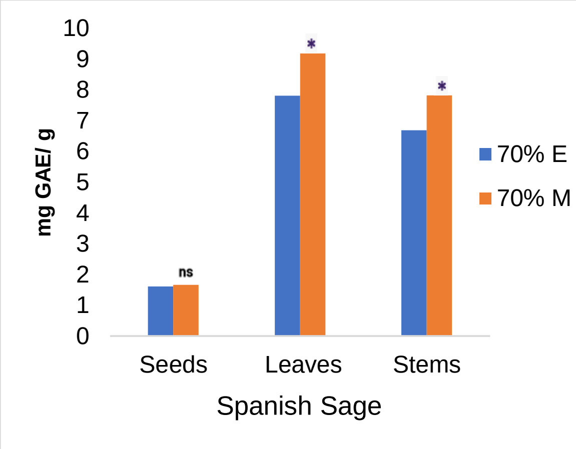

Figure 1 – Total phenolic content (as mg of gallic acid per 1.0 g sample) in Spanish Sage seeds, leaves and stems, obtained from extraction 70% ethanol (E) and methanol (M).
* Isstatically significant ($p < 0.05$), and ns,is no significant difference ($p > 0.05$)

Рисунок 1 – Общее содержание фенолов (в мг галловой кислоты на 1,0 г образца) в семенах, листьях и стеблях испанского шалфея, полученных путем экстракции 70 % этанолом (E) и метанолом (M).
* Является статически значимым ($p < 0{,}05$), а ns – не имеет существенной разницы ($p > 0{,}05$).

Figure 1 shows that a higher total phenolic content was obtained by 70 % methanol extraction of various parts of the Spanish sage plant (seeds, stems and leaves) compared with ethanol extraction: the result for seeds, leaves and stems of the plant was 0.055; 1.374 and 1.124 mg GAE/g of the extract. Moreover, it was found that the total content of phenolic compounds has the highest value in methanol extracts from plant leaves (9.183 ± 0.0625 mg GAE/g of extract), which exceeds the studied indicator in stems by 1.364 and in seeds by 7.514 mg GAE/g of methanol extract, respectively.

3.2. Antioxidant activity

The results of the antioxidant activity of chia seeds obtained in this research (41.35 ± 0.2%) are shown in Figure 2 andit is in harmony with the DPPH radical scavenging activity of *Salvia hispanica* species such as Salvia caespitosa, 41.3%; *Salvia hypargeia,* 34.6%; and *Salvia candidissima* subsp. *Candidissima,* 49.7% reported by Tepe et al., [28].

The DPPH radical scavenging activity of the Spanish sage plant leaves (92.24 ± 0.3%) is in accordance with the DPPH radical scavenging activity of the Lamiaceae family has already been described in species such as Salvia officinalis (92.3 ± 0.5%), Salvia sclarea (92.9 ± 0.4%), *Salvia glutinosa* (91.5 ± 0.5%) and Salvia pratensis (93.0 ± 0.5% IDPPH) by Miliauskas et al., [29]. Additionally, this result is 1.5-fold higher than the inhibition percentage reported by Zuniga-Lopez et al. [30]. The antioxidant activity of Chia plant stem was for the first determined (74.43 ± 0.3%). Generally, the DPPH radical scavenging activity of chia SSL showed high inhibition percentage.

Natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C)[31][32].The high antioxidant capacity of chia SSL detected could be attributed to the presence of phenolic compounds. Our result was in harmony with the previous report like Kahkönen et al., [33] showed variation in phenolic content between different parts of different trees.

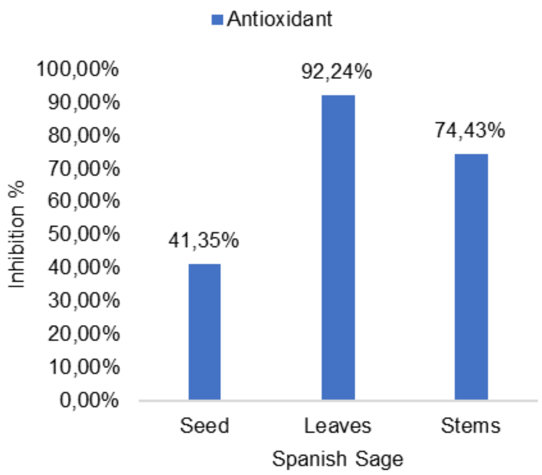

Figure 2 – Antioxidant capacity of chia SSL extracts
Рисунок 2 – Антиоксидантная способность экстрактов семян чиа

Figure 2 shows the highest antioxidant activity in the leaves of the Spanish sage plant (92.24%), surpassing this indicator in the stems of the plant by 17.81% and chia seeds by 50.89%.

4. Conclusions

The result of the chia plant (Spanish Sage seeds, leaves and stems) introduced to Russian Federation, which was grown in southern forest-steppe of Western Siberia shows high phenolic content and antioxidant capacity. The results exhibited that the phenolic content in *Salvia hispanica L.* seeds can be extracted with ethanol and methanol solutions. The ethanolic extract and methanolic extract of chia leaves and stems showed significant difference, while for the chia seeds there was no significant difference. Furthermore, in this study the leaves and stems showed higher TPC and antioxidant activity as compared to other edible plants and in comparison, with in the Spanish Sage plants, green leaves quantified higher TPC and antioxidative activities followed by chia stems and chia seeds. Thus, this study supplies new information of the chia SSL introduced in the Russian federation about the main phenolic content present in chia products, which are important dietary sources of natural antioxidants for prevention of diseases caused by oxidative stress. The phenolic compounds found in these samples may decrease the invasiveness of cancer cells, remove ROS (reactive oxygen species) and improve the clinical outcome. Nevertheless, it is necessary to evaluate the antioxidant activity against oxidative injury including absorption mechanism of the phenolic compounds in vitro and in vivo assays. As indicated with the results of chia seeds in combination with yogurt in our pervious study reported by L.A. Nadtochii et al., [34], these Spanish Sage plantseeds, stems and leaves can be used as ingredients for functional food and for food supplements production due to the high bioactive properties. Moreover, the consumption of chia seeds can be an important alternative to improve consumer's health, and the results obtained suggest its use as a functional food in human daily diet.

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