



33(41A): 167-174, 2021; Article no.JPRI.72325 ISSN: 2456-9119 (Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Phytochemical Screening and Antioxidant Activity of Salvia hispanica

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Authors' contributions

This work was carried out in collaboration among all authors. Author HD designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors HD and MS managed the analyses of the study. Authors FZB, and MF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i41A32314 <u>Editor(s):</u> (1) Dr. S. Prabhu, Sri Venkateswara College of Engineering, India. <u>Reviewers:</u> (1) Galina Ignatieva Kaz-Pektin Too, University of Murcia, Spain. (2) Adou Marc, University of Nangui Abrogoua, Côte d'Ivoire. Complete Peer review History: <u>https://www.sdiarticle4.com/review-history/72325</u>

Original Research Article

Received 05 June 2021 Accepted 10 August 2021 Published 17 August 2021

ABSTRACT

Background: There is a growing interest worldwide to identify novel functional foods able to exert important biochemical activities with low or without toxic effect. Accordingly, the use of Salvia hispanica L. (chia) a Mexican herb, has tremendously grown as an agriculture crop owing to the nutritional and medicinal values. The objective of this study was to evaluate phytochemical composition of chia seeds and prove their claim for functional properties.

Materials and Methods: The determination of total phenolic, flavonoid and condensed tannin contents of chia seeds were carried out by the Folin-Ciocalteu, the aluminum trichloride and the vanilline acid spectrometric methods. Antioxidant activities of chia seeds extracts were assessed using free radical scavenging assay DPPH, Ferric Reducing Antioxidant Potential FRAP and β -Carotene bleaching assay.

Results: *Chia seeds contain high levels of total phenolic and flavonoid contents* 19.06 \pm 0.14 mg GAE/ g DW and 12.3 \pm 0.04 mg CE/g DW, respectively. The content of condensed tannins was estimated at 8.32 \pm 0.01 mg CE/g DW. The flavonoid extract showed a higher antioxidant potential against DPPH, FRAP and the bleaching of β -Carotene (0.27 \pm 0.00, 0.06 \pm 0.03 and 0.39 \pm 0.01 mg/mL, respectively).

Conclusion: Following the obtained results, we should explore the use of this seeds in food products due its nutritional and chemical composition, possible human health benefits and role as a functional food.

Keywords: Salvia hispanica L.; Chia ; phenolic compounds; antioxidant activity.

1. INTRODUCTION

Chia (Salvia hispanica L.) is an annual herbaceous plant belonging to Lamiaceae family. It is originated from Southern Mexico and Northern Guatemala and can grow in the arid and semi-arid regions of the world due to its low water requirements [1,2,3]. As reported in the literature, chia today is not only cultivated in Mexico and Guatemala, but also in Australia, Bolivia, Columbia, Peru, Argentina, America, and Europe. Nowadays, Mexico is recognized as the world's largest chia producer [4]. Although different parts of the plant have been used for pharmaceutical feeding, medicinal, and purposes, the most attractive part of the plant is the seed [5], which has been a part of human diet since 3500 BC [6].

The chia seed is an oilseed that is considered as a pseudo-cereal with a powerhouse composed of fats, carbohydrates, dietary fiber, proteins, vitamins (A, B, K, E, D), minerals, and antioxidants which is cultivated for different usage and commonly grown in several countries [7].

Seed is small with an oval, slightly flattened shape and may have a color ranging from white to black [6]. It has been directly consumed as whole grain or grounded in refreshing drinks and salads. It has been also added as an ingredient in the production of many bakeries such as bread, cakes, and cookies [8].

In recent years, Chia seeds have become one of the world's most recognizable foods based on their nutritional properties and medicinal values [9,10,11]. The advantages of using seed as a nutritional supplement are enormous positive benefits include supporting the digestive system, promoting healthy skin, stronger bones and muscles, reducing the risk of heart disease, diabetes, and so on [12,13]. These healthpromoting effects are attributed to its constituents, mainly to its oil, dietary fibers, and antioxidant compounds; the seeds are free from mytoxins and it does not contain gluten [14,5].

In this study the interest is based on chia seeds (*Salvia hispanica* L.) and aimes to quantify the

phenolic compounds contained in the hydromethanolic extract and to evaluate the antioxidant properties of extracts separately by diffrent methods.

2. MATERIALS AND METHODS

2.1 Plant Materials

Commercial chia seeds imported from Argentina and packed in polypropylene bags were purchased from a super market (Tlemcen, Algeria). Chia seeds were ground to obtain powder that were further used for analysis.

2.2 Preparation of Extracts

2.2.1 Total phenolic compounds

The phenolic extract was obtained according to the method of Jimoh et al. [15]. Briefly, 10 g of the ground chia seeds were extracted with methanol/water, (80/20, v/v) by maceration at room temperature for 24 hours. Then the extracts were filtered through filter Whatman 0.5 paper under vacuum. The filtrate was concentrated to dryness under reduced pressure at 45°C, and then redissolved in 10 mL of methanol. The methanolic extract was stored at 4°C, for further investigations.

2.2.2 Total flavonoid compounds

10 g of dried material were extracted with 100 mL of methanol (MeOH) and 5 g of carbonate of Calcium by boiling for 1 h [16]. After filtration with Whatman 0.5 paper, the MeOH was evaporated under reduced pressure using Buchi R-200 rotavapor. Subsequently, recover the dry extract with 50 mL of boiling water. The aqueous extract was filtered and then fractionated by liquid – liquid extraction, first with ethyl acetate and with n-butanol, using a separating funnel. All the fractions were concentrated and kept at 4°C.

2.2.3 Total Tannin compounds

As described by Bruneton et al. [17], five grams of powdered material (chia seeds) were extracted at 4°C using 200 ml of a mixture of acetone/water (25/45, v/v) for 96 hours. After

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filtration with whatman 0.5 paper, acetone was evaporated under reduced pressure. Subsequently, the dichloromethane $(2 \times 25 \text{ ml})$ was used for the extraction of lipids and pigments. Afterward, the aqueous phase was extracted with 25 ml of ethyl acetate. This process was repeated 4 times. The organic phases were finally gathered and evaporated to dryness at 40°C, weighed and dissolved in 3 mL of methanol.

2.3 Determination of Phenolic Compounds

2.3.1 Total phenolic content (TPC)

TPC of chia seeds extract was determined using Folin–Ciocalteu reagent as described by [18]. Briefly, 20 μ L of the extract was diluted with 1580 μ L of distilled water, followed by addition of 100 μ L of Folin–Ciocalteu reagent (2N) and 300 μ L of Na₂CO₃ solution (7.5%). The mixture was incubated for 2 hours at room temperature in the dark followed by measuring the absorbance at 760 nm using a spectrophotometer Specord ^R 200 Plus UV/Vis. Gallic acid was used to construct the standard curve and the results were expressed as mg gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

2.3.2 Total flavonoid content (TFC)

TFC was determined according to Djeridane et al. [19]. In brief, 1 mL of extract was mixed with 1 mL of methanolic $AlCl_3$ (2%). After incubation at room temperature for 15 min, the absorbance was measured at 430 nm using a spectrophotometer Specord ^R 200 Plus UV/Vis. The content of flavonoids was determined with reference to standard curve determined with catechin, and expressed as mg catechin equivalents (CE) per grams of dry weight (mg CE/g DW).

2.3.3 Condensed tannin content (CTC)

CTC were determined according to Julkunen-Titto [20]. 50 μ L of the extract was added to 1500 μ L of vanilline/methanol (4%, m/v), followed by addition of 750 μ L of concentrated hydrochloric acid and allowed to react at room temperature for 20 min. the absorbance was measured against a blank at 550 nm using a spectrophotometer Specord ^R 200 Plus UV/Vis. The content of condensed tannins was determined with reference to standard curve determined with catechin, and expressed as mg catechin equivalents (CE) per grams of dry weight (mg CE/g DW).

2.4 Antioxidant Activity

2.4.1 DPPH free-radical-scavenging assay

Fifty (50) microliters of various extracts concentrations were added to 1950 μ L of 0.025g/L DPPH methanolic solution. After 30 min of incubation at room temperature, the absorbance was measured against a blank at 515 nm [21]. DPPH free radical scavenging activity percentage (RSA) was calculated using the following formula:

$$RSA(\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the negative control; A_{sample} is the absorbance of tested sample.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plotted graph of inhibition percentages against extract concentrations. The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as positive control.

2.4.2 Ferric reducing antioxidant potential (FRAP) assay

After adding 1 mL of each extract at different concentrations to 2.5 mL of 0.2 M phosphate buffer at pH= 6.6 and 2.5 mL of a 1% potassium ferricyanide solution, the obtained mixture was incubated for 20 minutes at 50°C, and then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. The mixture was centrifuged at 650g for ten minutes at room temperature and 2.5 mL of the supernatant were added to 2.5 mL of distilled water and 0.5 mL of 0.1% iron chloride [22]. The absorbance was read at 700 nm against a blank. The results make it possible to calculate the effective concentration (EC_{50}) . which is the extract concentration corresponding to an absorbance equal to 0.5, the linear regression curve (optics density as a function of the different concentrations). The extract activity was finally compared with the positive control 6hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox).

2.4.3 β-Carotene bleaching inhibition capacity

Antioxidant activity based on the β -carotene/ linoleic acid method was evaluated by measuring the inhibition of the bleaching of β -carotene by the peroxides generated during the oxidation of linoleic acid. Two mg of β-carotene were dissolved in chloroform (20 mL). 4 mL of this solution were added to linoleic acid (40 mg) and Tween 40 (400 mg). Chloroform was evaporated under vacuum at 40 °C and 100 mL of oxygenated water were added. An emulsion was obtained by vigorous shaking. A 150 µL of emulsion was placed in a microtiter plate well containing a 10 µL aliquot of each extract concentration [23]. The absorbance of each well at 470 nm was recorded twice, at 0 min and at 120 min after being incubated at 50°C. The antioxidant activity of the extracts was evaluated in terms of bleaching inhibition of the β-carotene emulsion using the following formula:

$$\beta carotene \ bleaching \ inhibition \ (\%) = [(A_{S120} - A_{C120}) \\ /(A_{C0} - A_{C120}) \times 100]$$

Where A_{C0} and A_{C120} are the absorbance of the negative control at 0 and 120 min, respectively, and A_{S120} is the sample absorbance at 120 min. The results were expressed as IC_{50} values. The Gallic acid was used as positive control.

3. RESULTS AND DISCUSSION

Chia seed as part of the human diet has started to gain increasing attention and been considered as a functional food due to its high nutritional value and health-promoting effects attributed to its constituents.

The estimation of total phenolic, flavonoid and condensed tannin contents of chia seeds were carried out by the Folin-Ciocalteu, the aluminum trichloride and the vanilline acide spectrometric methods. The results are expressed successively in mg gallic acid equivalent and mg Catechin equivalent per g of dry weight (Table 1). According to the results obtained the chia seeds exhibited high total phenolic contents with (19.06±0.14 mg GAE/g DW), represented mainly by flavonoids (12.3±0.04 mg CE/g DW). In addition, the lower content is attributed to condensed tannins (8.32±0.01 mg CE/g DW). These results are overall higher than found by Tuncil et al. [24], Marineli et al. [14] and Martinez-Cruz & Paredes-Lopez [25]. They reported the total phenolic content of Argentinian chia seeds to be 3.5±0.07 mg GAE/ g defatted chia seeds. Chilean chia seeds to be 0.94±0.06 mg GAE/ g sample, and Mexican chia seeds to be 1.64±0.08 mg GAE/ g sample, respectively. Also, Oliveira-Alves et al. [26] reported that the total phenolic compounds in commercial sample of chia seeds was found in crude extract about 1.16 mg GAE/g.

The differences in total phenolic content between our study and the others could be due to two factors; i) the samples used in our study and in the others are harvested from different locations, and it has been proven that growing locations significantly impact the composition of chia seeds [1,27,28,29]; ii) the methods used for the extraction of phenolic compounds in different studies vary, and different extraction methods have been shown to dramatically influence the total phenolic contents of chia seeds measured [30].

Focusing on phenolic content, dry chia seeds contain 8.8% of phenolic compounds. Besides that, high levels of caffeic acid, chlorogenic acid, querencetin, rosmarinic acid, gallic, cinnamic and ferulic acids, myricetin, quercetine, kaemferol, epicatechin, rutin, apigenin and p-coumaric acid are also reported. Furthermore, isoflavones, such as daidzein, glycitein, and genistein, are found in small amounts [4]. Studies also confirmed chia seeds are a rich source of particularly interesting groups of phytocompounds characterised by high biological activity [25,31-32]. Rahman et al. [33] reported that rosmarinic acid and daidzein are the major components found in chia seeds, along with caffeic acid, mycertin, quercetin. The flavonoids quercetin, chlorogenic acid, and caffeic acid are proven to have anticancerogenic, anti-hypertensive, and neuron protective effects [11]. Furthermore, Uribe et al. [34] and Reyes-Caudilio et al. [35] described that chia seeds are a great example of a food rich in antioxidants. with a wide range of antioxidant compounds like dietary fiber, essential fatty acids as well as polyphenols [36], phenolic compounds constitute the largest group of secondary plant metabolites in plants possessing key properties for their adaptation to the environment [37]. As bioactive substances, they are potent antioxidants and free radical scavengers widely used and requested in food, pharmaceutical and cosmetic industries [38].

In the same way, chia seeds have very good antioxidant activity as depicted by DPPH, FRAP and β -Carotene assays as well as calculating the IC₅₀ and EC₅₀ values. Results are shown in (Table 2).

Chemical constituent	Phenolic	Flavonoid	Condensed Tannins	
	(mg GAE/g DW)	(mg CE/g DW)	(mg CE/g DW)	
Chia seeds	19.06±0.14	12.3±0.04	8.32±0.01	
mg GAE/g DW: mg of gallic acid equivalents per g of dry Weight. mg CE/g DW: mg of catechine equivalents per				

|--|

g of dry Weight. The data are displayed with mean ± standard deviation of triplicate

Chia seeds extracts	DPPH IC ₅₀ (mg/mL)	FRAP EC ₅₀ (mg/mL)	β-Carotene IC ₅₀ (mg/mL)
TP	1.38±0.18	0.10±0.007	1.04±0,05
FLEA	0.27±0.00	0.06±0.03	0.39±0.01
FLnb	0.45±0.01	0.16±0.05	1.26±0.04
TN	1.50±0.09	0.80±0.01	0.41±0.06
Trolox	0.15±0.04	0.30±0.00	-
Gallic acid	-	-	0.43±0.00

Table 2. Antioxidant activity of chia seeds extracts

TP: total phenolic, FlvEA: Flavonoid ethyl acetat, Flvnb: Flavonoid n-butanolic, TN: tannins. The data are displayed with mean ± standard deviation of triplicate replications, Mean values followed by different superscript in a column are significantly different (P= .05).

In DPPH assay, compounds which are able to donate hydrogen or electron to DPPH are considered as antioxidants and therefore radicals scavengers. The efficiency of extracts was determined as the lowest concentration providing 50% of inhibition. The results shown in Table 2 reveal that flavonoid ethyl acetate extract exerted the highest DPPH scavenging potential with an IC₅₀ of 0.27±0.00 mg/mL. These results are far from those reported by Tuncil et Celik [24] where metanolic extract of Argentinian chia seeds revealed significant potential with IC₅₀ of 2±0.01mg/mL. The higher antioxidant activity of chia seeds could be attributed to the higher total phenolic contents (Table1).

In FRAP assay Ferric Reducing Antioxidant Potential, as shown in Table 2, the flavonoid etylacetat extract revealed the best iron reducing power with an EC₅₀ of 0.06±0.03 mg/mL, higher than Trolox with 0.30±0.00 mg/mL and even higher than total phenolic and flavonid nbutanolic extracts 0.10±0.007 and 0.16±0.05 mg/mL, respectively.

In β -Carotene bleaching inhibition capacity, the less B-carotene discoloration rate is the more effective the extract is. Flavoind ethylacetat, tannins and Gallic acid had closer antioxidant activities with IC₅₀ about 0.39±0.01 and 0.41±0.06 and 0.43±0.00 mg/mL, respectively. Besides location effects, the differences between the findings could be attributed to the various techniques used for the extraction process, which have been shown to noticeably influence the final results of antioxidant activity of chia seeds [30].

Polyphenolic compounds are the most important complexes that contribute to the antioxidant activity of chia seeds. It is well known that they have the ability to scavenge free radicals, to chelate ions, and to donate hydrogens [10]. Their biological activities vary from antioxidant, antiaging, and anti-hypertensive to anti-cancerogenic and anti-inflammatory [4].

Several studies provided evidence for the high antioxidant potential of chia seeds. Sargi et al. [39] showed that chia seeds are capable of deactivating ABTS cation radicals, scavenging synthetic DPPH radicals and reduce iron ions. Results obtained in both tests indicate a higher antioxidant activity of chia seeds in comparison to flaxseed. Antioxidant activity of chia seeds was also confirmed by Coelho & Salas-Mellado [40] and Martinez-Cruz & Paredes-Lopez [25]. They showed that extracts from chia seeds are able to scavenging DPPH radicals and they cause their neutralisation by over 70%.

The same results were obtained by other authors such as Ciau-Solis et al. [41] and Reves-Caudillo et al. [35]. Reves-Caudillo et al. [35] investigated the antioxidant activity of phenolic compounds in chia seeds from two different regions in Mexico. The ABTS+ radical scavenging method, together with β -carotene linoleic-acid principle and phospholipid liposome peroxidation, was used in research to determine antioxidant activity, whilst Guindani et al. [42] used the ABTS+ method to determine antioxidant activity as well. Alacantara et al. [43] investigated antioxidant activity by the DPPH method. These results highlight that chia seeds are an important dietary source of natural antioxidants with possible effects on oxidative stress.

4. CONCLUSION

Already thousands of years ago, chia seeds were a staple food and were taken by pre-Columbian community. In recent years, there has been considerable interest in this raw material due to its high nutritive value. Our study confirms that the chia seeds exhibit high contents of phenolic compounds with antioxidant activity, effectively suggesting that Chia can may provide health benefits when used in food products.

CONSENT

It is not applicable.

ETHICALAPPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: https://www.sdiarticle4.com/review-history/72325