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First Report on Cultivated *Salvia hispanica* in an Arid Climate: UPLC-MS/MS Analysis, Antioxidant, and Enzymatic Activities

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Abstract. This study is the first to identify polyphenols and flavonoids in ethanolic extracts of aerial parts of *Salvia hispanica* cultivated under arid conditions using UPLC–MS analysis. Antioxidant activity was evaluated through DPPH, ABTS, PHE, and FRAP tests, while enzymatic activity was assessed via α-amylase inhibition. The polyphenol and flavonoid contents were highest in the inflorescences, followed by the leaves and stems. The major compounds identified included oleanolic acid, riboflavin,

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rutin, naringenin, curcumin, caffeic acid, resveratrol, quercetin, epicatechin, and luteolin. The chelating activity of leaf, inflorescences, and stem extracts ranged from 11.97 to 145.86 μg mL^{-1}, while α-amylase inhibitory activity ranged from 7.66 to 21.25 μg mL^{-1}. These findings highlight the pharmacological potential of *S. hispanica*, particularly its antioxidant and antidiabetic properties. This research enhances scientific understanding of Chia's aerial parts, supporting the development of plant-based supplements and medicinal applications for health and wellness.

Resumen. Este estudio es el primero en identificar polifenoles y flavonoides en extractos etanólicos de las partes aéreas de *Salvia hispanica* cultivadas en condiciones áridas mediante análisis UPLC-MS. La actividad antioxidante se evaluó mediante pruebas DPPH, ABTS, PHE y FRAP, mientras que la actividad enzimática se evaluó mediante inhibición de la α-amilasa. Los contenidos de polifenoles y flavonoides fueron más altos en las inflorescencias, seguidos de las hojas y los tallos. Los principales compuestos identificados incluyeron ácido oleanólico, riboflavina, rutina, naringenina, curcumina, ácido cafeico, resveratrol, quercetina, epicatequina y luteolina. La actividad quelante de los extractos de hojas, inflorescencias y tallos varió de 11.97 a 145.86 μg mL^{-1}, mientras que la actividad inhibidora de α-amilasa varió de 7.66 a 21.25 μg mL^{-1}. Estos hallazgos resaltan el potencial farmacológico de *S. hispanica*, en particular sus propiedades antioxidantes y antidiabéticas. Esta investigación profundiza la comprensión científica de las partes aéreas de la chía, impulsando el desarrollo de suplementos vegetales y aplicaciones medicinales para la salud y el bienestar.

Introduction

Salvia hispanica, a member of the Lamiaceae family and commonly known as Chia, is an annual herbaceous plant native to Mexico and Guatemala [1]. It was domesticated around 3500 B.C. and, alongside maize, beans, and amaranth, remained a staple crop until the arrival of the Spaniards [2]. It naturally grows in tropical regions (from 0 °C to 20 °C) and subtropical areas (from 20 °C to 40 °C). It is capable of thriving in arid conditions, which provides it with resistance to sudden droughts caused by climate change [3].

Chia seeds are rich in omega-3 fatty acids, fiber, protein, and both micro and macro minerals. As a result, they have been used in human food for centuries. Furthermore, its vegetative parts have been utilized for both nutritional and therapeutic purposes since ancient civilizations [4–8]. It has gained popularity in health food circles since the early 21st century, with products such as tablets, oil, yellow seed powder, bars, puddings, juices, and yogurt, due to their health benefits [9,10]. In addition to being considered a superfood, Chia seeds are used for medicinal purposes due to their high content of phenolic compounds, including caffeic acid, gallic acid, ferulic acid, depsides (such as rosmarinic acid and chlorogenic acid), and flavonoids (quercetin, myricetin, kaempferol, and apigenin). These compounds contribute to various significant biological activities, including antioxidant, antibacterial, anti-inflammatory, and antidiabetic properties [11–21]. Despite the growing interest among researchers in the seeds, reports on the aerial and root parts of the Chia plant remain limited [22–27], revealing a knowledge gap that requires further investigation.

From an ecological perspective, climate change is one of the most significant challenges agriculture is currently facing. As a result, selecting tolerant cultivars that can adapt to harsh environmental circumstances is necessary. Within the framework of this discussion, the significance of cultivating plant species with low water requirements, such as *S. hispanica*, was brought to light. This species is an excellent option because it is tolerant to drought and generates substantial biomass [28].

On January 15, 2023, the first experiment of cultivating Chia seeds (*Salvia hispanica*) was conducted in Algeria, in the El Outaya area of Biskra province, which is recognized as an arid region with unique climatic conditions, as part of initiatives to enhance agriculture and diversify crops. This study is the first investigation to assess the polyphenol and flavonoid content in ethanolic extracts of the aerial parts (leaves, inflorescences, and stems) of *S. hispanica* under arid conditions and to identify the bioactive compounds using UPLC- MS apparatus.

Experimental

Chia (*S. hispanica*) was cultivated for the first time in Algeria, in the El-Outaya region of Biskra Province (29°59'19"N, 1°42'57"W, 87 m) in a greenhouse (unheated and non-climate-controlled) used solely for physical

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protection against frost, wind, and sandstorms (Fig. 1). In January, daytime temperatures reached up to 17 °C, while in the cooler months, they ranged between 16 °C and 25 °C. Relative humidity remained low throughout the period (20–60 %), and no rainfall occurred. The soil was sandy and poor in organic matter.

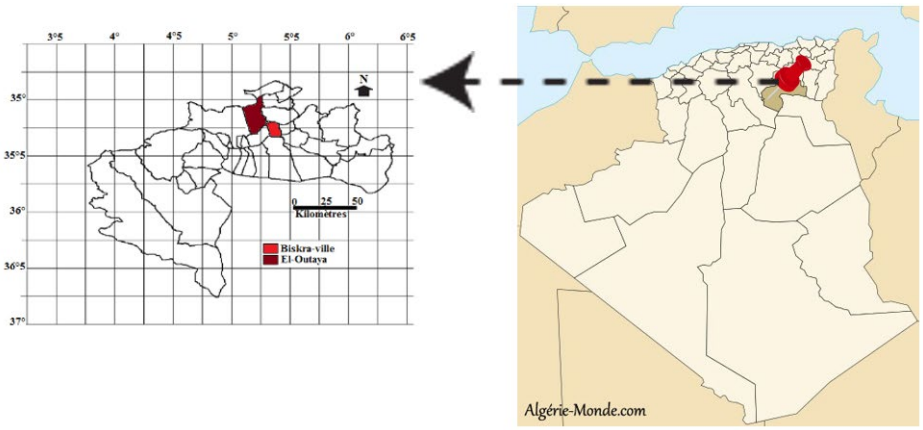

Fig. 1. Map of the experimental area of *S. hispanica* plant cultivation in the El-Outaya region (Biskra).

Plant material and sample preparation

The aerial parts (leaves, inflorescences, and stems) of *S. hispanica* were collected during the flowering stage on March 15, 2023, and dried at room temperature (approximately 24 °C). They were then ground using a Sayona-type coffee grinder (350 W). Ten grams of the powdered aerial parts (leaves, flowers, and stems) were extracted with 100 mL of ethanol using the ultrasonic extraction method (Sonics Vibra-cell VCX500) for 30 minutes at 15 °C. The mixtures were filtered using Whatman filter paper. The ethanolic extracts were then concentrated at 40 °C using a rotary evaporator (Büchi R-210 System) apparatus.

UPLC-ESI-MS/MS analysis

The analytical technique used to determine the bioactive compounds of ethanolic extracts from S. hispanica aerial parts is a Shimadzu 8040 Ultra-High Sensitivity UPLC-ESI-MS/MS system, equipped with a binary Pump, Nexera XR LC-20AD. The electrospray ionization (ESI) source was operated in both positive and negative ionization modes, and the collision-induced dissociation (CID) gas was set to 230 kPa. The conversion dynode voltage was -6.00 kV. The desolvation line (DL) temperature was maintained at 250 °C. The nebulizing gas flow was established at 3.00 L min-1, with the heat block at 400 °C and a drying gas flow of 10 L min-1. The mobile phase consisted of solvent A (water with 0.1 % formic acid) and solvent B (methanol), with a flow rate of 0.2 mL min-1. A volume of 5 µL was injected into a Restek Ultra C18 column (3 µm, 150 x 4.6 mm) to achieve effective analyte separation at ambient temperature. The elution was performed in gradient mode as follows: 2–55 % B over 2.5 min, 55–95 % B over 4 min, maintained at 95 % until 7 min, then returned to the starting conditions at 7.1 min and held for 5 min to ensure column equilibration.

Compound identification was performed using multiple reaction monitoring (MRM), tracking specific transitions from precursor to product ions. When available, analytical standards were used to confirm compound identities. In cases where standards were not available, identification was based on spectral data from the NIST 2020 mass spectral database. For quantitation, the most intense transition per compound was selected. Dwell times and collision energies were optimized individually to ensure the highest selectivity and sensitivity.

Determination of total phenolic content (TPC)

The total phenolic contents of *Chia* plant aerial parts were measured using the Folin–Ciocalteu reagent, following the procedure published by Müller et al. [29]. Gallic acid was used as a positive control, and the TPC was calculated and expressed as the sample's µg of gallic acid equivalents per mg (µg GAE mg-1 DE).

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Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) of the samples was assessed using the aluminum chloride ($AlCl_3$) colorimetric method [30]. The absorbance was recorded at 440 nm. Quercetin was used as a standard, and the TFC was calculated and expressed as µg of quercetin equivalents per mg (µg EQ mg-1).

DPPH free radical scavenging assay

The antioxidant capacity of the aerial parts of Chia was evaluated using the DPPH assay as described by Bouchoukh et al. [31]. The absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control.

ABTS free radical scavenging assay

The ability of ABTS to scavenge free radicals was assessed using the procedure outlined by Re et al. [32]. Absorbance was recorded at 734 nm. BHT was used as a standard control.

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity test was assessed using the protocol outlined by Oyaizu [33], with absorbance recorded at 700 nm. Ascorbic acid was used as a positive control.

Phenanthroline assay

This assay was conducted according to the protocol described by Szydłowska-Czerniak et al. [34], with absorbance measured at 510 nm. BHT was used as a positive control.

The α-Amylase inhibition activity

α-amylase inhibition activity was determined using the procedure described by Zengin et al. [35]. The absorbance was measured at 630 nm.

Statistical analysis

The results of the antioxidant activity are presented as means ± SD (N = 3). One-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$), was done to calculate the significance of difference, using GraphPad Prism 7.

Results and discussion

Determination of total phenolic content (TPC)

Polyphenols are a group of bioactive compounds found in plant products, including fruits, vegetables, tea, and coffee. They are recognized for their antioxidant, antidiabetic, anticancer, and anti-inflammatory effects, illustrating their ability to address various mechanisms that lead to chronic diseases. The results showed that the inflorescence extract had the highest amount of polyphenols, at 176.18 ± 2.31 µg GAE mg-1, followed by the leaf extract, at 135.44 ± 5.12 µg GAE mg-1, and the stem extract, at 59.23 ± 4.56 µg GAE mg-1 (Table 1). Our results are consistent with a study on the effect of the drying method on polyphenol content before and after drying of Chia herb [23]. However, our findings were higher than those reported for the methanolic extract of Chia seeds [36]. In comparison with other salvia species, our results were higher than those studied in ethanolic extracts of aerial parts of S. nemorosa, S. verticillata, S. nutans [37], and S. officinalis, and S. sclarea [38].

Determination of total flavonoids content (TFC)

Flavonoids are chemical compounds that belong to the class of polyphenols, such as quercetin, rutin, and luteolin, which were detected in our samples. The findings showed that the inflorescence extract had the highest content of flavonoids, with 42.49 ± 1.50 µg QE mg-1, followed by the leaf extract with 29.22 ± 1.36 µg QE mg-1, and then the stem extract with 18 ± 2.50 µg QE mg-1 (Table 1). Our findings are consistent with a similar study of

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extracts of S. lanigera and S. aegyptiaca, which reported 35.68 ± 1.84 and 40.63 ± 2.11 mg g-1, respectively [39]. However, our results were higher than those of a study on S. balansae [40].

Table 1. Total polyphenol and flavonoid content in ethanolic extracts of the aerial parts of *S. hispanica*.

Extracts	Inflorescences	Leaves	Stems
TPC (µg GAE mg^{-1} dry weight extract)	176.18 ± 2.31	135.44 ± 5.12	59.23 ± 4.56
TFC (µg QE mg^{-1} dry weight extract)	42.49 ± 1.50	29.22 ±1.36	18 ± 2.50

Chemical analysis using UPLC-ESI- MS/MS

The phytochemical analysis of ethanolic extracts from the leaves, inflorescences, and stems of Salvia hispanica revealed a diverse set of bioactive compounds, including flavonoids, phenolic acids, terpenoids, vitamins, and carotenoids (Tables 2–4). The distribution and abundance of these compounds varied considerably among the plant parts. In the leaf extract (Table 2, Fig. 2), the major compounds detected were riboflavin (relative peak area = 2,235,892), oleanolic acid (6,601,402), naringenin (461,453), and rutin (365,549). These results highlight the leaf's richness in flavonoids and vitamins, which are known for their antioxidant and anti-inflammatory potential. The inflorescence extract (Table 3, Fig. 3) displayed a broader chemical diversity, with 13 compounds identified. Among them, oleanolic acid (3,541,058), riboflavin (2,147,077), and rutin (1,093,692) were the most abundant. Additional compounds such as caffeic acid, epicatechin, quercetin, sinapic acid, and curcumin were also detected, supporting the extract's high antioxidant capacity. The stem extract (Table 4, Fig. 4), although less diverse (6 compounds), contained notably high levels of oleanolic acid (7,377,293) and riboflavin (23,453,647), suggesting that stems may serve as a potent source of terpenoids and vitamins. Overall, the results demonstrate apparent compositional differences: inflorescences were the richest in phenolic and flavonoid compounds, terpenoids dominated stems, while leaves offered a balanced profile of various bioactive classes. This suggests that different plant parts of S. hispanica may be selectively targeted depending on their intended biological application antioxidant, anti-inflammatory, or antidiabetic. Naringenin, quercetin, luteolin, sinapic acid, and caffeic acid were identified in the methanolic extract of the leaves [41]. In another investigation [26] to examine the profile of secondary metabolites of Chia leaves of two phenotypes (black and white Chia seeds), caffeic acid was detected in the ethanol and ethyl acetate extracts. At the same time, the dimethyl quercetin was found in ethyl acetate and dichloromethane extracts. In addition to the bioactive compounds previously reported in the literature on aerial parts of the Chia plant [25, 26, 41], riboflavin (vitamin B2), resveratrol, and oleuropein were identified for the first time in our study.

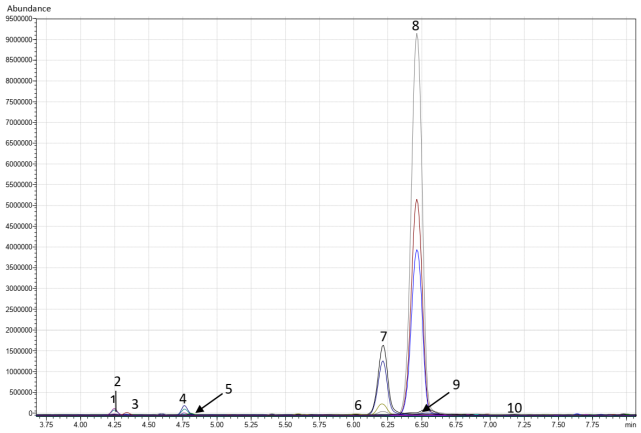

Fig. 2. UPLC-ESI-MS/MS chromatogram illustrating the bioactive compounds identified in the leaf extract of *S. hispanica*: 1 = Rutin; 2 = Quercetin; 3 = Resveratrol; 4 = Naringenin; 5 = Luteolin; 6 = Oleuropein; 7 = Oleanolic acid; 8 = Riboflavin; 9 = β-Carotene; 10 = Curcumin.

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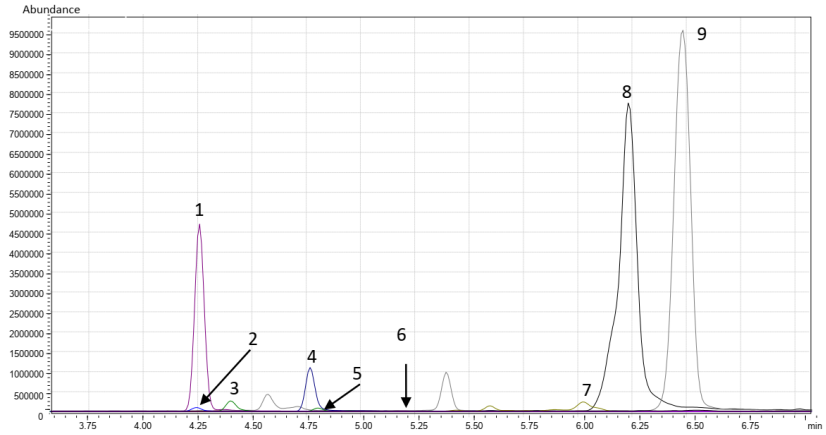

Fig. 3. UPLC-ESI-MS/MS chromatogram illustrating the bioactive compounds identified in the inflorescences extract of *S. hispanica*: 1 = Rutin; 2 = Quercetin; 3 = Resveratrol; 4 = Naringenin; 5 = Luteolin; 6 = Sinapic acid; 7 = Oleuropein; 8 = Oleanolic acid; 9 = Riboflavin. Other compounds (caffeic acid, epicatechin, β-carotene, and curcumin) are not shown due to their low abundances.

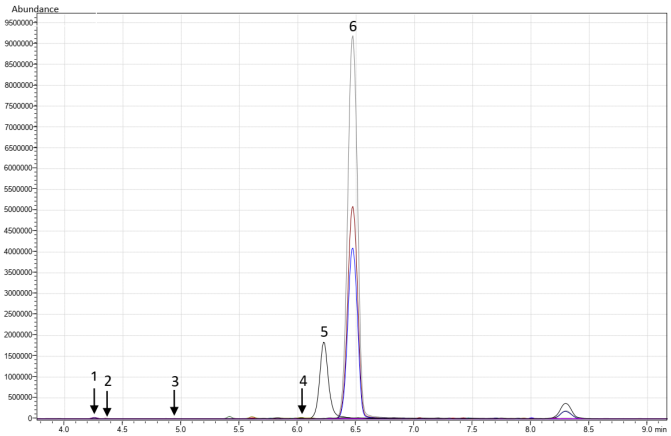

Fig. 4. UPLC-ESI-MS/MS chromatogram illustrating the bioactive compounds identified in the stems extract of *S. hispanica*: 1 = Rutin; 2 = Resveratrol; 3 = Luteolin; 4 = Oleuropein; 5 = Oleanolic acid; 6 = Riboflavin.

Table 2. Bioactive compounds identified in the ethanolic extract of chia leaves using UPLC-ESI-MS/MS.

Compound	**t_R**	**Formula**	**$[M+H]^+$**	**$[M+H]^-$**	**MS^2**	**Area %**
Rutin	4.248	$C_{27}H_{30}O_{16}$	611.0	-	465.20, 303.10, 85.10	365549
Quercetin	4.251	$C_{15}H_{10}O_7$	303.05	-	153.10, 137.10, 69.05	7672
Resveratrol	4.339	$C_{14}H_{12}O_3$	229.05	-	135.10, 107.10, 91.10	80355
Naringenin	4.763	$C_{15}H_{12}O_5$	273.05	-	153.00, 147.10, 91.10	461453
Luteolin	4.809	$C_{15}H_{10}O_6$	286.75	-	153.00, 135.00, 69.10	75755
Oleuropein	6.021	$C_{25}H_{32}O_{13}$	-	540.50	523.40, 88.950, 71.00	63350

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Oleanolic acid	6.218	$C_{30}H_{48}O_3$	457.30	-	439.35, 411.40, 81.00	6601402
Riboflavin	6.465	$C_{17}H_{20}N_4O_6$	377.10	-	360.30, 342.35, 297.15	22358892
β-Carotene	6.536	$C_{40}H_{56}$	537.10	-	505.00, 282.05, 280.95	240184
Curcumin	7.196	$C_{21}H_{20}O_6$	368.90	-	285.15, 177.05, 145.05	13111

Table3. Bioactive compounds identified in the ethanolic extract of chia inflorescences using UPLC-ESI-MS/MS.

Compound	**t_R**	**Formula**	**$[M+H]^+$**	**$[M+H]^-$**	**MS^2**	**Area %**
Caffeic acid	3.840	$C_9H_8O_4$	-	179.15	135.00, 134.00, 89.05	9531
Rutin	4.248	$C_{27}H_{30}O_{16}$	611.0	-	465.20, 303.10, 85.10	10936928
Quercetin	4.251	$C_{15}H_{10}O_7$	303.05	-	153.10, 137.10, 69.05	140838
Resveratrol	4.339	$C_{14}H_{12}O_3$	229.05	-	135.10, 107.10, 91.10	19735
Naringenin	4.763	$C_{15}H_{12}O_5$	273.05	-	153.00, 147.10, 91.10	2139247
Luteolin	4.809	$C_{15}H_{10}O_6$	286.75	-	153.00, 135.00, 69.10	552242
Epicatechin	5.124	$C_{15}H_{14}O_6$	290.80	-	165.15, 139.15, 123.15	17780
Sinapic acid	5.199	$C_{11}H_{12}O_5$	225.00	-	207.15, 175.01, 91.00	34501
Oleuropein	6.021	$C_{25}H_{32}O_{13}$	540.50	-	523.40, 88.950, 71.00	811647
Oleanolic acid	6.218	$C_{30}H_{48}O_3$	457.30	-	439.35, 411.40, 81.00	35410508
Riboflavin	6.465	$C_{17}H_{20}N_4O_6$	377.10	-	360.30, 342.35, 297.15	21470771
β-Carotene	6.536	$C_{40}H_{56}$	537.10	-	505.00, 282.05, 280.95	58047
Curcumin	7.196	$C_{21}H_{20}O_6$	368.90	-	285.15, 177.05, 145.05	45017

Table 4. Bioactive compounds identified in the ethanolic extract of chia stems using UPLC-ESI-MS/MS.

Compound	**t_R**	**Formula**	**$[M+H]^+$**	**$[M+H]^-$**	**MS^2**	**Area %**
Rutin	4.248	$C_{27}H_{30}O_{16}$	611.0	-	465.20, 303.10, 85.10	88186
Resveratol	4.339	$C_{14}H_{12}O_3$	229.05	-	135.10, 107.10, 91.10	51762
Luteolin	4.809	$C_{15}H_{10}O_6$	286.75	-	153.00, 135.00, 69.10	9272
Oleuropein	6.021	$C_{25}H_{32}O_{13}$	540.50	-	523.40, 88.950, 71.00	65130
Oleanolic acid	6.218	$C_{30}H_{48}O_3$	457.30	-	439.35, 411.40, 81.00	7377293
Riboflavin	6.465	$C_{17}H_{20}N_4O_6$	377.10	-	360.30, 342.35, 297.15	23453647

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Antioxidant activity

The analytical data on the antioxidant activity of the ethanolic extract of the aerial parts of *S. hispanica* are presented in Table 4. The values were reported as IC_{50} for the DPPH and ABTS tests and $A_{0.5}$ for the FRAP and phenanthroline tests. Ascorbic acid and BHT served as positive controls. The phenanthroline assay demonstrated that Chia plant leaves exhibited the highest chelating capacity, with an $A_{0.5}$ value of 11.97 ± 0.25 µg mL^{-1}, which was significantly comparable to BHT ($A_{0.5}$ = 9.71 ± 0.9 µg mL^{-1}). This was followed by inflorescences with a value of 42.57 ± 1.28 µg mL^{-1}, while stems showed the lowest chelating activity at 145.86 ± 11.73 µg mL^{-1}. In the DPPH assay, the degree of color change (from purple to yellow) is used to measure the antioxidant activity of the tested sample. A more significant decrease in purple chromogen indicates higher antioxidant activity, as the tested sample, such as the plant extract or synthetic compound, effectively neutralizes the DPPH radical by donating electrons or hydrogen atoms. The plant extracts of Chia parts showed strong antioxidant activity in the following order: Inflorescences with an IC_{50} value of 64.79 ± 1.03 µg mL^{-1} compared to BHT, which has an IC_{50} value < 12.5 µg mL^{-1}. Followed by leaves of 107.67 ± 0.96 µg mL^{-1} and stems of 150.45 ± 1.78 µg mL^{-1}. The decrease in absorption of the ABTS radical in the presence of antioxidants indicates higher antioxidant activity in the tested sample, reflecting their ability to neutralize peroxyl radicals (ROO·) and thus protect against oxidative stress. Based on the results (Table 5), the best scavenging ability was recorded in Chia inflorescences with an IC_{50} value of 41.68 ± 0.23 µg mL^{-1}, comparable to the BHT with an IC_{50} value < 12.5 µg mL^{-1}, then the leaf with a value of 136.06 ± 1.40 µg mL^{-1}. In contrast, the scavenging activity in stems was weak, with a value of 422.09 ± 21.08 µg mL^{-1}. The reducing power assay is a standard method to assess the antioxidant potential of natural extracts or other substances by evaluating their ability to reduce ferricyanide ions, which reflects their electron-donating capacity and, thus, their ability to act as antioxidants. The inflorescence extract exhibited significant reducing power, with an A0.5 value of 71.35 ± 0.38 µg mL^{-1}, compared to the standard ascorbic acid, which showed a value of 6.52 ± 0.07 µg mL^{-1}, followed by the leaf extract at 156.23 ± 0.51 µg mL^{-1}. However, the stem extract showed no activity at a concentration of 200 µg mL^{-1}. This activity could be attributed to the abundance of biologically active molecules such as terpenoids (oleanolic Acid), riboflavin (vitamin B2), phenolic acids (curcumin, caffeic acid, sinapic acid, and resveratrol), and flavonoids (rutin, naringenin, quercetin, epicatechin, and luteolin).

In comparison to other exciting literature on the antioxidant capacity of salvia species, which has confirmed their effectiveness in reducing or destroying the effect of free radicals in the body, our results are comparable, for instance, to a study on determination of the bioactive compounds and antioxidant activity of ethanolic extracts of sage aerial parts collected from Transylvanian region (Romania) including *S. austriaca, S. sclarea*, and S. *pratensis* [42]. Similarly, trend for *S. austriaca* and *S. nutans* [43]. Oleanolic acid has several biological activities, including its antioxidant effect by influencing GSH (glutathione) levels and antioxidant enzymes and exhibiting metal chelation activity [44]. Riboflavin (vitamin B2) is an essential vitamin that plays a crucial role (as an antioxidant) in the body. It supports the function of the enzyme glutathione peroxidase and the production of NADPH, which enhances the body's ability to combat oxidation and protect cells from the resulting damage [45].

Table 5. *In vitro* antioxidant activity of the aerial parts of *S. hispanica*.

Extract	DPPH IC_{50}(µg/mL)	ABTS IC_{50}(µg/mL)	PHE $A_{0.5}$ (µg/mL)	FRAP $A_{0.5}$ (µg/mL)
Leaves	107.67± 0.96^{b}	136.06 ± 1.40^{b}	11.97 ± 0.25^{a}	156.23± 0.51^{b}
Inflorescences	64.79± 1.03^{a}	41.68 ± 0.23^{a}	42.57 ± 1.28^{b}	71.35±0.38^{a}
Stems	150.45 ±1.78^{c}	422.09 ± 21.08^{c}	145.86 ± 11.73^{c}	>200
BHT	< 12.5	< 12.5	9.71 ± 0.9^{a}	-
Ascorbic acid	-	-	-	6.52±0.07

Different lowercase letters indicate significant differences according to Tukey's test at $P < 0.05$.

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In vitro enzyme inhibitory activity

Alpha-amylase is crucial for breaking down carbohydrates, such as starches, into simple sugars. It is primarily secreted in the saliva and pancreas, where it initiates the digestive process. It has been shown that alpha-amylase inhibitors can treat obesity and diabetes [46]. As shown in Fig. 5, the extracts from the stems of *S. hispanica* exhibited high inhibitory activity with an IC_{50} value of 7.66 ± 0.25 μg mL^{-1}, which is very close to that of the reference inhibitor, acarbose (IC_{50}= 6.96 ± 0.4 μg mL^{-1}). The leaf extract showed the second-best inhibitory activity of 11.76 ± 0.59 μg mL^{-1}, followed by the inflorescence extract, which showed the least activity of 21.25 ± 0.68 μg mL^{-1}. Based on the area of the identified peak (the one that has been recognized) compared to the total area of only the identified peaks, the high activity observed in the stems in inhibiting the α-amylase enzyme could be attributed to its composition, which is mainly comprised of riboflavin (75.5% of the identified compounds) and oleanolic acid (23.76 % of the identified compounds). In the leaves extract, riboflavin constitutes 73.87 %, while oleanolic acid represents 21.81 %. In contrast, the percentages in the inflorescences were 29.97 % for riboflavin and 49.42 % for oleanolic acid, respectively. Additionally, this high activity may be attributed to unidentified bioactive compounds. Only 17 references were used to identify phenolic compounds, resulting in a limited number of compounds being identified. Therefore, other unidentified bioactive compounds may contribute to the observed enzymatic inhibition in the stems.

Our findings demonstrated remarkable efficacy compared to other chemical studies; for instance, a recent study examined the antidiabetic activity of the dichloromethane fraction obtained from the aerial parts of the plant, yielding a biological value of IC50 of 673.25 μg mL^{-1} [24]. However, it is essential to note that studies specifically addressing α-amylase inhibition in S. hispanica seeds are limited, underscoring the need to explore other plant parts. For example, a study utilizing LC-HR/MS profiling of chia seed oil reported relatively low inhibitory activity (IC_{50} = 553.07 μg mL-1) compared to the acarbose standard, with an IC_{50} value of 7.54 μg mL^{-1}, likely due to the oil's non-polar composition [47]. Similarly, whole seed extracts exhibited moderate activity (IC_{50} = 121.46 μg mL^{-1}) [48]. In contrast, our previous investigation on the organic fraction of chia seeds revealed good activity (IC_{50} = 34.97 ± 0.68 μg mL^{-1}), suggesting that extraction selectivity plays a key role in enhancing bioactivity [21]. Moreover, while several other Salvia species such as *S. officinalis, S. macilenta, S. mirzayanii, S. santolinifolia* [49], *S. aegyptiaca*, and *S. verbenaca* [50] have exhibited notable α-amylase inhibitory activity in previous studies, the aerial parts of *S. hispanica* evaluated in this study demonstrated even stronger effects. The abundance of compounds such as riboflavine, oleaolic acid, rutin, quercetin, and oleuropein, known for their biological activity, may play a role in this high enzymatic activity. Riboflavin has been shown to inhibit α-amylase and improve metabolic disorders in certain patients. It is recommended as part of daily meals and has potential as an adjunct treatment for diabetics, as well as protection against diabetes risk [51]. Resveratrol is believed to exert its effects on amylase through different mechanisms. Antioxidant properties reduce the enzyme's catalytic capability; resveratrol can bind to the surface of amylase to inhibit enzymatic activity. Lowering amylase activity helps improve blood glucose levels, thus offering therapeutic potential for obesity and metabolic syndrome [52]. Rutin has potent antioxidant activity *in vivo* and *in vitro*. This biological activity helps protect the human body from degenerative diseases due to oxidative stress. Furthermore, this flavonoid possesses some unique traits; it enhances antioxidant activity and gives feedback to the body, awakening the antioxidant system and inducing various phase II detoxifying enzymes. Additionally, rutin can negatively interact with certain medicinal compounds that are metabolized and transported by specific proteins. However, recent research has revealed that rutin possesses another biological activity: the ability to inhibit the function of the alpha-amylase enzyme [53, 54]. Quercetin strongly reduces free radicals in the body and enhances antioxidant activity. In addition to inhibiting the enzyme alpha-amylase, which contributes to reducing glucose absorption in the intestine and regulates blood sugar levels, it is a promising ingredient in managing diabetes [55]. Oleanolic acid can inhibit the alpha-amylase enzyme [44]. Several studies have reported the alpha-amylase, alpha-glucosidase, and pancreatic lipase inhibitory activities of oleuropein using *in vitro* assays. The antioxidant and enzyme-inhibitory properties of oleuropein were confirmed. However, these properties must be further evaluated to determine the mechanisms of oleuropein and related health benefits [56].

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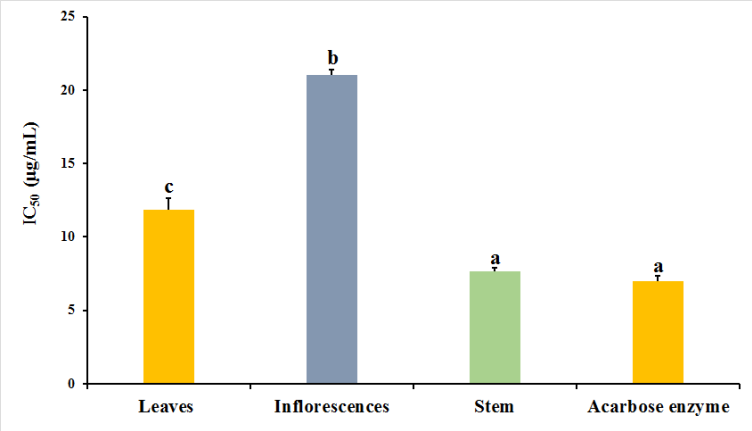

Fig. 5. *In vitro* enzymatic activity of the aerial parts of *S. hispanica*. Data presented are mean ± standard deviation. Different lowercase letters indicate significant difference according to Tukey's test at alpha =0.05.

Conclusions

In conclusion, this study provides new insights into enzyme activity. It shows the remarkable antioxidant activity of the crude ethanolic extracts of the aerial parts of the Chia plant grown in the EL Outaya Biskra region, Algeria, due to their richness in bioactive compounds such as riboflavin, oleanolic acid, rutin, resveratrol, quercetin, and oleuropein, which were identified using the UPLC-MS technique. Additionally, the plant extracts presented excellent activity in inhibiting the enzyme alpha-amylase, thus improving the control of diabetes levels. This study suggests that the Chia plant is a promising natural source in the pharmaceutical industry for treating and controlling chronic diseases.

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