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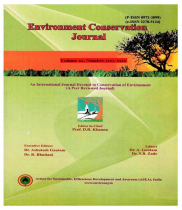

Morpho-quantitative and biochemical characterization of Chia (*Salvia hispanica* L.) seeds to understand its benefits and to increase its adaptability

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ABSTRACT

Chia (*Salvia hispanica* L.), of the mint family Lamiaceae, is one of the most highly nutritious crops in the world. It has a high economic value in both national and international markets. The present study was carried out with the prime objective of assessing Chia's morphological, biochemical, and nutritional characterization. An average yield of Chia (784 kg/ha) from the field experiment was observed. The biochemical studies showed the presence of higher amounts of carbohydrates, phenols, flavanols and antioxidants. The seed protein content of Chia was evaluated, and it was found to be 183 mg/g, which was greater than other major crops like wheat, rice and maize. The Carbohydrate content (371 mg/g) was also high in Chia seeds. High amounts of phenols (1.29 mgGAE/g) and flavonoids (0.48 mg/g) in Chia seeds were also observed. The mineral content estimated by ICP-OES showed the presence of micronutrients like Fe (11.7 mg/100g), Mg (335 mg/100g), Mn (5.97 mg/100g), Zn (12.01 mg/100g), Cu (1.94 mg/100g), Ca (397.78 mg/100g), Na (42.15 mg/100g) and K (605.83 mg/100g). The FTIR analysis showed the presence of the functional groups, and high peak banding was found related to protein, pectin (polysaccharides), PUFA (fatty acids), lipids etc. The HPTLC analysis indicated the presence of Gallic acid. Thus the present study unveils that the seeds of the Chia crop are a rich source of different essential elements. Hence this pseudo-cereal Chia can be used to provide good food supplements. As this is a newly introduced crop in India, there is very less study on the crop. To utilize the benefits of this crop, further research in various aspects to increase the environment adaptability and yield should be done.

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Introduction

Chia (*Salvia hispanica* L.) is a herbaceous plant belonging to the order Lamiales, the mint family Lamiaceae and the genus Salvia. The genus name Salvia is derived from the Latin word called "Salvare," which in Latin means "to heal" or "to be safe and unharmed," referring to the medicinal properties of the genus.

This genus's origins have been traced back to Afghanistan and Soviet Central Asia. Mexico has approximately about 250 species (Jamboonsri *et al*., 2012). Chia seeds have been used as a food source since 3500 B.C. Chia seeds are consumed as the main grain alone or in combination with other cereals, and they are also used for a variety of medicinal purposes. Chia is a pseudo-cereal, raised for its edible and highly nutritious seeds. It is being called a "Superfood" by the nutritional community due to its high nutritional value. Under ideal agronomic conditions, the plant can produce 500-600 kg of seed per acre (Ullah *et al*., 2016).

The high nutritional and pharmacological value of Chia seed (Munoz *et al.,* 2013) has increased interest among researchers in exploring opportunities for the species to be grown outside of these areas. Compared to other oilseeds or cereal seeds, Chia seeds have a 19-23% high protein content. Methionine and cysteine are particularly abundant in the seed flour protein fraction (Ixtania *et al.,* 2008). For adding to the health benefits of Chia seeds, one cup contains 34 to 40g of fiber, which equals 100% of the daily recommendation for adults to reduce the risk of coronary heart disease. Chia seeds are also a source of antioxidants such as caffeic acid, chlorogenic acid, kaempferol, and quercetin, providing many significant health benefits. Besides this, it reduces insulin resistance, improves blood sugar level, reduces inflammation, and provides vital nutrients for bone health (Webmd, 2022). In addition, plenty of macronutrients like phosphorous and micronutrients like copper, iron, manganese and molybdenum are also found in Chia seeds (Beltan-Orozco and Romero, 2003).

Therefore, this study aims to conduct morpho-quantitative analysis, phytochemical analysis and antioxidant activity analysis of Chia seeds to understand the crop's benefits and increase its environment adaptability.

Material and Methods

Collection of plant materials and Morpho-quantitative trait assessment

Chia Seeds were collected from the All India Coordinated Research Network on Potential Crops (AICRN on Potential Crops), OUAT, Bhubaneswar. The field experiment was carried out at its experimental station. The seeds were sown in the field for recording the morphological observations during the *Kharif* season of 2020 with a spacing of 45x10 cm having a plot size of 5.0 sq. meter. All the recommended agronomic practices were implemented in raising a good crop and were harvested. Observations were recorded taking five random plants selected from the middle rows of the plot for nine morpho-quantitative traits from days to flower to days to attain maturity and 10ml seed weight, which were recorded on a plot basis and from a random sample of the plot, respectively and flowering days were recorded considering all the plant present in the plot.

Preparation of seed extract and Biochemical analysis

The ground sample (1.5 g) was extracted using an environmental shaker after adding 20 ml of 80% methanol placed under room temperature for a night and centrifuged for 15 min at 8000 rpm and a temperature of 4°C. The supernatant was collected and dried. The weight of Chia seed extract was measured and then made up with methanol at 1mg/ml (Beltran-Orozco *et al.,* 2020). Di-acid digestion for mineral estimation was carried out using a 3:2 mixture of HNO_3:$HClO_4$. As the sample was high in fats/oils, pre-digestion using 10 ml HNO_3 /g sample was carried out to avoid explosion (Zasoski and Burau, 1977). The carbohydrate content was obtained using the Anthrone method, whereas protein content was calculated using the Lowry method. The total phenolic content of the Chia seed extracts was determined by comparing them with the standard antioxidants like Gallic acid according to the Folin–Ciocalteu method (Gamez-Meza *et al.,* 1999).

One ml Folin–Ciocalteu reagent was added to the extract. Then 2 ml of 10% Sodium carbonate was added after 5 mins and kept aside at room temperature for 2 hrs. Chia seed extracts and standards readings were measured at 660 nm against distilled H_2O using a UV

spectrophotometer. A calibration graph of the standard Gallic acid is prepared, and the results were calculated using it and expressed as Gallic acid equivalents (GAE mg/g). For protein extraction, two grams of seed powder were homogenized in 5ml of 10% TCA using a pre-chilled mortar pestle. The content was incubated overnight at 4ºC and centrifuged at 8000 rpm for 10mins. First, pellets were washed to remove the pigments with 2ml of 100% acetone. Next, pigment-free pellets were washed with 80% ethanol first and then by ethanol: chloroform (3:1) and washed by ethanol: ether (2:1) to eliminate the phenolic compounds.

Washed pellets were lyophilized and suspended in protein extraction buffer and boiled for 2mins, centrifuge at 12000 rpm for 10mins. The supernatant was taken for quantitative analysis. First, the total flavonoid content was measured by using the colorimetric method (Zhishen *et al.,* 1999) with some modifications. Here, 500 µL of chia seed extract was combined with 1000 µL of distilled water in a test tube, and then 75 µL of sodium nitrite ($NaNO_2$) 5% solution was added. After 5 minutes, 150 µL of a 10% $AlCl_3$ solution was added, and the mixture was agitated for another 5 minutes before adding 500 µL of 1M NaOH and 775 µL of distilled water. The absorbance of the sample was recorded immediately at a wavelength of 510 nm in a spectrophotometer and compared with a catechol calibration curve. The results were determined by calculating as gms of catechol equivalent in a kg of dry sample (g/kg equivalent dry sample).

The analysis was done according to the method described by Shimada (1992) for estimating antioxidant activity. The approach was based on the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging principle. First, the standard of BHT (synthetic antioxidant) was prepared at 500µg/ml to compare the antioxidant activity. Next, solutions of the Chia seed extracts and synthetic antioxidant BHT used in the study were prepared in methanol at concentrations of 50, 100,150 and 200 µg/mL and free radical scavenging activities were determined. Then, 1ml 0.004 % DPPH was added to the Chia seed extract and BHT standard solutions and mixed. After 30 minutes in the dark, the absorbance of each mixture was measured at 517 nm against a methanol blank. For calculation, the following formula was used.

$$\textbf{Antioxidant activity} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{absorbance of control}}$$

For mineral content estimation, the digested Chia seed sample volume consisted of distilled water to 50ml. The solution of the sample was then filtered with the help of Whatman no 42 filter paper. Finally, this digested Chia seed sample was used to calculate the amount of Fe, Mg, K, Ca, Mn, Zn, Na and Cu using the ICP-OES unit.

For FTIR analysis, the plant extract was made by centrifuging 2 gm of ground seed with 20ml of methanol, and the supernatant was used. The absorbance was taken at the wavenumber range of 4000-400 cm^{-1} The Gallic acid content determination was done (Kaya *et al.,* 2012). The reference standard used in the present study was Gallic acid (1mg/ml). All Chia seed extract samples were taken at a 1 mg/ml concentration. The 200 ml of mobile phase was taken in a ratio of 5:4:1containing toluene, ethyl acetate and formic acid. The TLC plate of analytical chromatography grade of 10 x 10 cm was used. Plates were evaporated to remove excess humidity at 70°C in a hot air oven for 10 minutes. 10µl each of sample and reference standard were spotted on silica plate and bands were observed by UV light at 254 nm.

Results and Discussion

The phenotypic study (Table 1) revealed quantitative characters like plot yield (0.279 Kg/plot), yield (7.84 qt/ha), 50% flowering (72 days), days of maturity (102 days), plant height (109.8 cm), no. of branches (13.2), no. of secondary branches (14), length of inflorescence (19.2 cm), length of primary inflorescences (9.2 cm), length of secondary inflorescence (2.8 cm), leaf length (7.49 cm) and leaf breadth (4.18 cm). The 10 ml seed weight was also calculated by measuring the number of seeds that could be packed in a 10 ml beaker, which was 6.05g. The present investigation revealed that phenolic compounds are present in high concentrations in seeds that provide antioxidant properties to the seeds. The seed protein content of Chia was evaluated and was found to

have significant protein content (183 mg/g). Carbohydrate content (371 mg/g) was also high in Chia seeds. Also, the phenol content (1.29 mg/g) and flavonoid content (0.48 g/100g) were high when compared to other cereals (Table 2).

Table 1. Morpho-quantitative data of Chia

Sl. No	Parameter	Mean value
1	Plant height(cm)	109.80
2	No. of branches	13.20
3	No. of secondary branches	14.00
4	Length of leaf(cm)	7.49
5	Breadth of leaf(cm)	4.18
6	Length of the main inflorescence(cm)	19.20
7	Length of primary inflorescence(cm)	9.20
8	Length of secondary inflorescence(cm)	2.80
9	10ml seed wt(g)	6.05
10	Yield/plant(g)	3.53

Table 2. Quantification of different biochemicals present in Chia seeds

Sl. No.	Parameter	Content
1	Carbohydrate(mg/g)	371
2	Protein(mg/g)	183
3	Phenol(mg/g)	1.29
4	Flavonoid (mg/g)	0.48

For antioxidant scavenging activity, different concentrations of Chia seed extracts were used in the DPPH assay and were compared with BHT (a synthetic antioxidant). The activities were as follows for 50µL (70.4%), 100 µL (71.9%), 150 µL (72.8%) and 200 µL (75.3%). The maximum activity was found when 200 µL of Chia seed extract was used (Table 3). The constituents were evaluated, and the findings are shown in Table 4.

From the plot data collected from the experimental station of AICRN on Potential Crops in the *Kharif* season of 2020, the yield per hectare was 784 kg/ha. The studies performed in Argentina have shown yields of 606 to 1400 kg/ha (Lobo *et al.,* 2011), whereas the studies conducted in Mexico showed a yield of 1200 kg/ha (Bochicchio *et al.,* 2015). The results of field experiments on Chia conducted in Germany gave yields of 618.39 to 1171.33 kg/ha (Grimes *et al.,* 2019). The biochemical analysis showed the presence of a high quantity of phenols in Chia seeds. The results showed the content of 1.29 mgGAE/g phenol in the Chia seeds. Furthermore, scientists researched the Chia seed chemical constituents and found the presence of phenols 0.97mg GAE/g seed sample (Beltan-Orozco *et al.,* 2020).

Martinez-Cruz and Paredes-Lopez (2014) calculated the amount of phenols in Mexican Chia as 1.63 mg GAE/g of seed sample. They recorded a total average of 0.66 to 0.9mg GAE/g for chia seeds cultivated in Mexico. In the present study, the amount of flavonoids in the Chia seed sample was calculated as 0.48 mg/g. The study revealed 0.36 mg/g of flavonoids in Chia seed samples (Beltran-Orozco, 2020). Scapin *et al.,* 2016, calculated the total flavonoids present in Chia seed extracts as 0.16 mg/g. In the present study for antioxidant scavenging activity, different concentrations of Chia seed extracts were used in the DPPH assay and were compared with BHT (a synthetic antioxidant). Maximum scavenging/inhibition activity was found when 200 µL of Chia seed extract was used and was about 75.3%. As a result of these findings, the chia seed sample had a high content of Fe (11.78 mg/g), Mg (>335 mg/g), Mn (5.97 mg/g), Zn (12.01 mg/g), Cu (1.94 mg/g), Ca (397.8 mg/g), Na (42.15 mg/g) and K (605.8 mg/g). Chia seeds showed a high quantity of iron, potassium and magnesium. In cells and body fluids, potassium regulates heart rate and blood pressure. When it comes to mineral abundance, magnesium was ranked sixth. There are two forms of this divalent cation in plants: bound and unbound. Magnesium content could not be calculated as magnesium concentration was saturated in the present Chia seed sample. Hence, it can be more than 335 mg/100g of Chia seeds. The transmittance bands show the functional groups present in the Chia seed sample. A better understanding of the chemicals present in Chia and their molecular analysis was carried out with the help of Fourier transform infrared (FTIR) technology. The bands were analyzed with the functional reference groups and identified the presence of different compounds in the Chia seed sample. The FTIR spectrum presented bands between 3700 cm^{-1} and 3000 cm^{-1},

Table 3. Antioxidant activity of chia seed extract.

Volume of sample (µL)	Chia absorbance	BHT absorbance	Scavenging activity (%) of Chia	Scavenging activity (%) of BHT
50µL	0.495	0.185	70.4	88
100µL	0.470	0.162	71.900	90.3
150µL	0.455	0.156	72.8	90.6
200µL	0.413	0.134	75.3	91.9

Table 4. Estimated mineral content (mg/100g) Chia seed sample.

Sl. No	Mineral	mg/100g-present study
1	Fe(238.204)	11.78
2	Mg(285.213)	>335
3	Mn(257.610)	5.97
4	Zn(206.200)	12.01
5	Cu(327.393)	1.94
6	Ca(317.933)	397.8
7	Na(589.592)	42.15
8	K(766.490)	605.8

N.B. Values within parenthesis represent absorbance in nm

Table 5. FTIR spectra of Chia seed and their corresponding annotations

Wavenumber (cm^{-1})	Transmittance (%)	stretches	Class of compounds	Intensity
3308.89	71.67	≡C-HO-HN-H	Alkynes	strong, sharp
			Amides	Weak to medium
			Alcohols	strong, broad
			Carboxylic acids	strong, broad
2943.86	79.07	C-H O-H	Alkanes	strong
			Alkyls	strong
			Carboxylic acids	strong, broad
2831.93	79.68	O-H	Carboxylic acids	strong, broad
2522.26	97.69	O-H	Carboxylic acids	strong, broad
1449.05	83.91	C=C, C-H	-CH2, Ester	
1115.59	88.45	C-FC-O-C	R-F(Alkyl fluoride)	very strong
			C=C-H2-OH	medium to strong
			C=C-CH(R-OH)	
			C=C-CRR'-OH	
1022.44	22.4	=C-O-C	Ether	Med to strong
			R-F	very strong
625.72	71.69	C-Br ,≡C-H	R-Br (Alkyl bromide)	strong
			Alkynes	strong, broad

Table 6. Rf values, Max % and Area % of Chia and Gallic acid

Sample	Start Rf	EndRf	Max%	Area%
Gallic acid	0.33	0.56	52.19%	60.82
Chia	0.41	0.53	6.23%	8.72

and the peaks between these bands may be related to the stretching of the O-H group's vibrations from hydroxyls of polysaccharides and proteins. The band at 3308 cm^{-1} shows amide stretch showing the presence of proteins (Table 5). A mobile phase with toluene, ethyl acetate and formic acid in the ratio of 5:4:1, respectively, was used in developing the HPTLC profile. Individual peaks were scanned at 254 nm to determine the Rf value and area unit (AU). The Rf values (Table 6) of the band for reference standard were: Gallic acid at Rf 0.33 – 0.56.

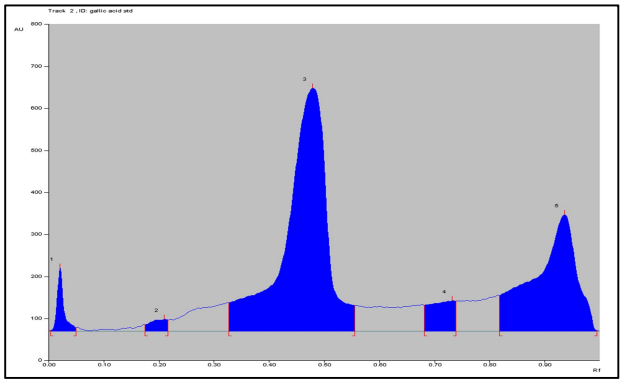

Figure 1. Graph showing the peak for Gallic acid.

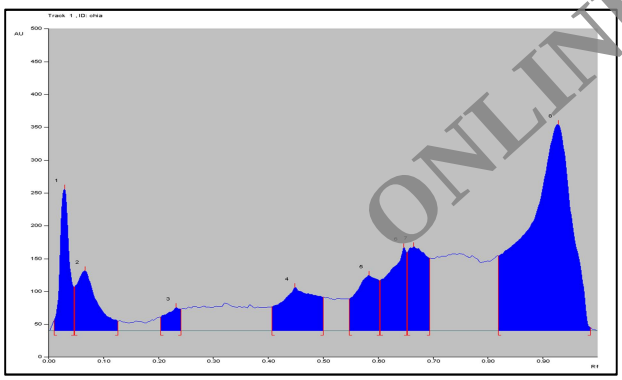

Figure 2. Graph showing the peak in the Chia seed samples comparable to the gallic acid peak.

The chemical components in the Chia sample extracts were detected by comparing bands in the sample with those of a reference standard on the same TLC plate. The HPTLC graph showed that Gallic acid was present in the Chia seed extract (Figure 1). Peak 3 of gallic acid was in the Rf range of 0.33 and 0.56, and a peak was obtained in the chia seed sample in the range of 0.41 and 0.53, which comes in the range of Gallic acid (Figure 2). So it was ascertained that the presence of phenols in the Chia seed extracts (the peak 4 in the Chia

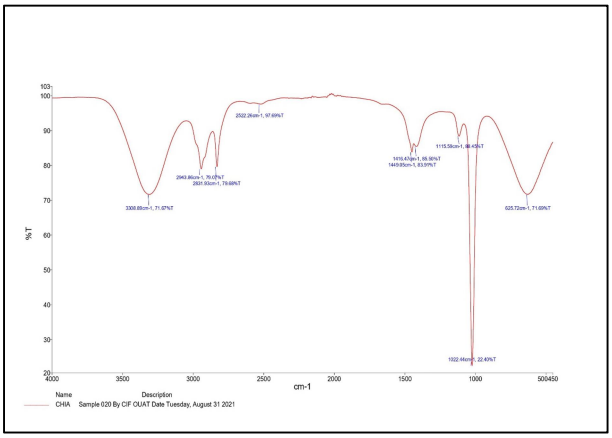

Figure 3. Spectral graph of functional group analysis of Chia seed extract.

sample was considered the Gallic acid). The -C-H stretches of aromatic rings and the methyl group signifying fat content were linked to the broad band at 2943.86 cm^{-1}. Transmittance at 1449.05 cm^{-1} shows the bending vibration of CH_2 groups (lipid) and ester carboxyl stretches of uronic acids in seed polysaccharides. The FTIR shows the presence of C=C bonds in high intensity that shows the presence of PUFA's (Polyunsaturated fatty acids) like alpha-linolenic acids. The high unsaturation in the spectra was caused due to alpha-linolenic acid present in the sample. The peak at 1115.59 cm^{-1} shows the presence of C-O-C (triglyceride ester linkage). At 1449 cm^{-1}, it shows the peaks for methyl esters in pectin. The FTIR results show the presence of high amounts of polysaccharides, lipids, triglycerides, polyunsaturated fatty acids (PUFA), and pectin (Table 5, Figure 3). At 1449 cm^{-1}, it shows the peaks for methyl esters in pectin. The FTIR results show the presence of high amounts of polysaccharides, lipids, triglycerides, polyunsaturated fatty acids (PUFA), pectin etc. HPTLC technique was highly used in pharmaceutical industries to separate new promising pharmaceutical compounds (Maripandi *et al.,* 2010). The HPTLC chromatogram of the seed extracts of Chia shows the presence of Gallic acid. The HPTLC profile was developed using a mobile phase/solvent system as toluene: ethyl acetate: formic acid (5:4:1). Peak 3 of gallic acid was in the Rf range of 0.33 and 0.56. A peak was observed in the chia seed sample in the range of 0.41 and 0.53, which comes in the range of Gallic acid, indicating the presence of the phenols in the

Chia seed extracts. Chia seeds' DPPH radical scavenging activity was 68.83 per cent (inhibition %). There was a strong antioxidant capacity because of the abundance of phenolic chemicals (Martinez-Cruz and Paredes-Lopez, 2014; Dash *et al.,* 2022). Chia seeds had a higher percentage of inhibition than the standard drug at 500 µg/ml (92% and 67%, respectively), as found by Dugganaboyana *et al.,* 2016. Biochemical analysis of the Chia seed showed the presence of high concentrations of protein, carbohydrates, phenols and flavonoids. The seed protein content of Chia was evaluated and was found a significant amount of protein. The protein content was 183 mg/g, i.e., 18.3%. Kasuya *et al.,* 2012 calculated 15-25% of the protein in Chia seeds. In comparison to other cereals, chia seeds have a protein level of 17 per cent (for example, in corn, the amount of protein content was 9.4 per cent, rice was 6.5 per cent, quinoa 14.1 per cent, and in wheat 12.6 per cent) (Knez *et al.,* 2019). Chia seeds' carbohydrate amount was 371 mg/g, i.e., 37.1%. The study conducted by Dugganaboyana *et al.,* 2016 showed that Chia seeds had 349.2 mg/g or 34.92 % of carbohydrates in Chia seeds. Chia seeds contain 42% of carbohydrates in them. Micronutrient elements present in Chia were evaluated after the wet digestion process (digestion using nitric acid and perchloric acid), followed by spectrometry by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) (USDA, 2018; Sahoo *et al.,* 2020). The different elements were analyzed, and the results were presented for the Chia seed powder was employed in this investigation.

Conclusion

Based on the current investigation, it is observed that the local collection of Chia seeds showed good adaptability with a significant quantity of yields. It is also found that the mineral content is higher with good sources of Fe, Mg, Mn, Zn, Cu, Ca, Na and K. Protein and carbohydrate content are also high. Chia crop is rich in phenols, flavonoids and antioxidant activity, which gives this crop a high medicinal value. The presence of high essential polyunsaturated fatty acids and polysaccharides in Chia is also observed. With good market potential and nutritional and medicinal value, this crop can be preferred to be grown on a commercial scale. The diversity, along with the amount of nutrient composition in chia seed, can help to have a healthy diet and add value to the preparation of products. However, in vivo and clinical studies on the safety and efficacy of chia seed are still limited. Although the presence of active ingredients in chia seed warrants its health benefits, the safety and efficacy of this medicinal food or natural product need to be validated by scientific research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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