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Original Article

The Chemical Composition of *Salvia macrosiphon* Seed

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Abstract

The Lamiaceae family of plants includes *Salvia macrosiphon*, one of the therapeutic plants. In Iran, *S. macrosiphon* seeds are used as a remedy to prevent or treat numerous diseases. The chia seed, also known as *Salvia hispanica*, has beneficial effects on human health. This study examines the chemical composition and nutritional value of *S. macrsoiphon* seeds. In some previous research, *S. macrosiphon* seeds components were compared with *Salvia hispanica*'s. In February 2021, *S. macrosiphon* seeds were bought from an herbal market; after they were authenticated, seed oil was obtained, and its chemical constituents were examined. *S. macrosiphon* seeds contain the following nutrients per 100 g: calcium 0.47 g, phosphorus 0.186 g, magnesium 9.75 g, moisture 5.2 g, ash 5.55 g, crude protein 18.92 g, crude fiber 22.2 g, carbohydrates 4.49 g, starch 17.25 g, total fiber 53.04 g, ADF (acid detergent fiber) 23.0 g, ADL (acid detergent lignin) 9.75 g, *S. macroiphon* seeds have 58.5059 calories total energy per gram. Fatty acids abound in *S. macrosiphon* oil, particularly linolenic (38.7%) and linoleic (24.5%) acids. Analysis and comparisons reveal that the chemical components obtained from *S. macrosiphon* and *S. hispanica* seeds are strikingly similar in quantity.

Keywords: *Salvia macrosiphon*; Chemical composition; Nutritional value; linoleic acids; crude protein; crude fiber.

1. Introduction

Salvia macrociphon Boiss (from the Lamiaceae family) is a perennial plant, almost greenish-white or yellow-green, slightly fragrant, covered with dense, non-tuberous fleecy, tuberous, and slightly hairy hairs at the ends. Its geographical distribution is in the west, central parts, south, southeast, and northeast of Tehran (Center of Iran) and its surroundings [1]. *Salvia macrosiphon* Boiss is a quite common and polymorphic plant in Iran and Afghanistan. It is a perennial herbaceous plant that has a lemon scent and is highly aromatic. Its stems are few to numerous from a woody rootstock, up to 60

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cm, tall, strong, quadrangular, below glandular pilose, above with a dense indumentum of short glandular hairs and sessile oil globules [2]. According to ethnopharmacological investigations, *S. macrosiphon* seeds are used as a medicine by Iran's indigenous people, and the plant's essential oil contains beneficial compounds, including linalool, hexyl isovalerate, hexyl hexanoate, hexyl-2-methyl-butanoate, hexyl octanoate, sclareol [3]. Also, in traditional medicine, the whole seeds of the plant are frequently boiled or soaked for inflammatory conditions. Documents have revealed little knowledge about the chemical components of these plant's seeds [4]. A study investigated the biological functions and phytochemical composition of *S. macrosiphon's* aerial parts. The antibacterial effects of the plant's n-hexane, chloroform, and ethyl acetate fractions on *S. aureus* and *E. coli* were tested, and the results showed good activity with MIC values ranging from 0.61 to 2.5 mg/mL. Numerous investigations continued to examine how cytotoxic activity worked. When tested against two separate human breast cancer cell lines and the lung cancer cell line A-549, the n-hexane fraction's efficacy was high (MCF-7 and MDA-MB-231). As a result, this fraction underwent its initial cytotoxic assessment and phytochemical investigation. It seems possible to use *S. macrosiphon's n*-hexane and chloroform fractions for thorough research to create an herbal anticancer [5]. The *S. macrosiphon* essential oil obtained by water distillation was studied using Gas Chromatography-Mass Spectrometry (GC-MS), and 30 compounds (about 90% of the components) were identified. Linalool (21.6%) and sclareol (15.7) were the two main ingredients [6]. In a study, the seed oil content and fatty acid (FA) profile of 21 populations from 16 wild Salvia species of Iran were analyzed by GC, and linolenic acid, linoleic acid, oleic, palmitic acid, and stearic acid in the plant seeds were identified and quantified [7]. A different study also looked at the aerial part of this plant yielded four flavonoids, namely apigenin-7, 4'-dimethyl ether, β-sitosterol, salvigenin, apigenin-7-O-glucoside, and luteolin-7-O-glucoside, and one steroid, namely apigenin-7. Using various chromatography and spectroscopic methods to isolate and identify these chemicals [8]. In another study, linalool was again listed as the major essential oil at 26.3% [9].

Similarly, *Salvia hispanica* L. is an herbaceous plant belonging to the Lamiaceae family and the genus Salvia (sage) (Lamiaceae). The primary industrial raw material extracted from *S. hispanica* is the seeds. Due to their high levels of dietary fiber and polyunsaturated fatty acids (PUFA), chia seeds are regarded as having great nutritional value. The seeds also include a variety of phenolic compounds, macro- and microelements, and vitamins in addition to healthy protein [10]. According to study findings, chia seeds help increase weight loss and reduce obesity-related risk factors while maintaining adequate glycemic control. Managing obesity in diabetes may benefit from adding chia seeds to the patient's diet and the standard medical care [11]. According to studies, chia seed oil possesses antidiabetic, anticancer, anti-inflammatory, anti-obesity, antioxidant, antihyperlipidemic, insect-repellent, and skin-healing qualities [12]. This study aims to evaluate the chemical composition and

nutritional value of *S. macrosiphon* (including polysaccharides, fatty acids, amino acids, minerals, dietary fibers, crude fiber, and phenolic compounds) with those of chia seeds. To the best of our knowledge, this is the first study to evaluate the composition of *S. macrosiphon* seeds and to compare them to chia seeds simultaneously.

2. Materials and Methods

2.1. Plant material

100 g *S. macrosiphon* seeds were obtained from an herbal medicine store in Tehran (February 2021). M. Kamalinejad from Herbarium in School of Pharmacy, Shahid Beheshti University of Medical Sciences approved seeds.

2.2. Ash, oil, and sugar determination

The dry matter content of the sample was calculated by placing the sample in an oven at 80°C for 24 hours. For Ash determination [12], 2 g of ground dried SM seed is added to the completely dry crucible and lid, and together, they are weighed to determine the mass of the sample by difference. The sample is placed in the hot furnace long enough so that complete combustion of the sample occurs. The crucible, lid, and ash are then re-weighed. Seed oil (crushed) was obtained by using the Soxhlet apparatus (100 mL) and *n*-hexane solvent at the rate of 24.40% [12]. The total sugar and starch in the seeds were calculated with an anthrone-sulfuric acid solution reagent. The amount of sugar and starch in the seeds in terms of glucose was obtained using standard glucose concentrations (drawing a standard curve) and with the same reagent using a UV spectrophotometer at 630 nm (using the accuracy and conversion factor) [13].

To extract sugars from test materials, we weighed approximately 0.2 g. of finely ground material into a 50-ml. Centrifuge tube: added two drops of 80% alcohol to aid mixing, and then 5 ml of water and stirred thoroughly. 25 mL of hot 80% alcohol was added, stirred thoroughly, set aside for five min., and centrifuged. After, an alcoholic solution was decanted, and repeated the alcohol extraction procedure by adding 30 mL of hot 80% alcohol to the residue. Two alcohol extracts were combined. Alcohol interfered with color development in the anthrone-sugar reaction and was removed from the combined extracts by evaporation under reduced pressure in a boiling water bath. The remaining cloudy aqueous fraction was diluted with water; a final concentration of sugars equivalent to about 100 pg. of glucose/ml. is required; the aqueous solution of sugars is then ready for analysis with the anthrone reagent.

Cell wall and non-cellular polysaccharides of *S. macrosiphon* seeds were analyzed by AOAC methods (001.43). Based on the methods of AOAC, dry matter, crude protein, crude fiber, crude fat, ash, sugar, and starch content were measured. Additionally, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by the methods [14]. The Megazyme assay kits (K-TDFR, Ireland) based on AACC (American Association of Cereal Chemists) method 32-07.01 and AOAC methods 991.43, were used to measure NSPs, soluble and insoluble fiber [15].

2.3. Crude protein determination

The amount of crude protein was obtained using the Kjeldahl method (1990 AOAC). The

1261 PARR calorimeter bomb measured gross energy (GE) [16]. The amount of seed phosphorus of the *S. macrosiphon* plant was obtained by measuring its absorption at a wavelength of 440 nm, called a spectrophotometer.

2.4. Mineral analysis

2g of seeds ground finely and ignited in a furnace to C-free ash. Once a few drops of HNo_3 had been added, the residue was boiled to being dried. FAAS used a 250 Plus Atomic Absorption Spectrometer (Varian, Australia) to determine the concentrations of Ca and Mg. The standard analytical parameters (air/acetylene flame) were utilized. Magnesium, calcium, and phosphorus absorption was read and measured at a wavelength of 285.2 nm, 422.7 nm, and 255 nm, respectively. The calibration curves of phosphorus, magnesium, and calcium were prepared using standards [17].

2.5. Fatty acid analysis

To analyze the fatty acid, a mixture of fatty acid standards (stearic acid, palmitic acid, linoleic acid, linolenic acid) was first prepared (from Merck Company) and then hydrolyzed and esterified with the methanolic Potassium hydroxide [14]. The mixture was injected into analytical gas chromatography, and its gas chromatogram was obtained. The same procedure was done with the oil extracted from the SM seed. At first, the seeds were crushed with an electric mill and were extracted in the Soxhlet apparatus. Then, the solvent (*n*-hexane was concentrated, and 1 mL of extract was mixed with methanolic Potassium hydroxide. Boron trifluoride was used as a catalyzer. Immediately, one microliter of the esterified fatty acid solution was injected into the Agilent 6890N Network Gas Chromatograph with a column (DB-FFAP). The temperature of the injection system was 230°C, and the detector temperature was 260°C. The column temperature program was 180°C, which reached a temperature of 230°C at a rate of 5°C. Nitrogen-carrying gas with a 2 ml/min flow rate was used, and the GC detector was FID [18]. Identifying fatty acids in SM seeds was based on comparing their retention times with esterified fatty acid standards.

2.6. Fiber determination

To determine the total fiber (TDF), a kit containing α-amylase, purified protease, and purified amylosidase (Megazyme International Ireland Ltd.) was used [16]. A fiber measuring device (Fibertec system 1010 heat extractor) was used to determine the amount of ADF (Acid Detergent Fiber) and NDF (neutral detergent fiber) [16, 19, 20].

2.7. Total phenolic determination

Measurements of total phenolic compounds and total tannins of plant seeds were performed using Folin Ciocalteu solution and tannic acid standard with a UV spectrophotometer at 725 nm [16, 20].

For this determination, the following was introduced into a 100 mL volumetric flask strictly in the order given: 1 mL of the extract, previously diluted 1:5, and 50 mL of distilled water. Then 5 mL of Folin Ciocalteu reagent was added, followed by 20 mL of sodium carbonate solution (anhydrous sodium carbonate, Na_2Co_3, made up into a 20 % w/v

solution). Finally, it made up the volume to 100 mL with distilled water. The content was then stirred to homogenize and was allowed 30 min for the reaction to stabilize. The absorbance at 725 nm is then read through a 1 cm cuvette and using a blank prepared with distilled water in place of the extract.

2.8. Amino acids determination

Seeds underwent an oxidation process (16 h with performic acid at 0°C, following this hydrolyzed (24 h with 6 N HCl at 110°C) to determine total amino acids. The hydrolysate sample was analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) [21]. Company Evonik Iran Ag did this work.

3. Results and Discussion

The analysis results of the seed compounds in the *S. macrosiphon* plant are presented in **Table 1**. The seeds have the content of dry matter (94.86%), total ash (5.55%), total protein (18.92%), total fat (24.40%), total energy (calories per gram 58.5059), calcium (0.47%), phosphorus (19.19%) and magnesium (9.75%).

The results of the analysis of cell wall and non-cellular polysaccharides of *S. macrosiphon* seeds are presented in **Table 2**. Cell wall polysaccharides per 100 g, had 53.04 g of crude fiber, 62.79 g of NSP (non-starch polysaccharides), 13.26 g of cellulose, 17.5 g hemicellulose, 22.20 g crude fiber, 40.50 g NDF (neutral detergent fiber), 23 g ADF (acid detergent fiber), 9.75 g ADL (acid detergent lignin), and non-wall polysaccharides per 100 g, including 17.25 g of starch, 4.49 grams of total free sugar and NFC (nanofibrillated cellulose) 9.38 grams. Because of their numerous physiological functions, particularly their significant antioxidant potential, polysaccharides have reportedly drawn much attention thus far in the research [22]. The amount of tannins and phenolic compounds in the seeds of the *S. macrosiphon* plant is shown in **Table 3**; the amount of tannins is 0.116%, and phenolic compounds 1.003%.

Table 1: Results of the *S. macrosiphon* seed composition analysis.

Magnesium (%)	Phosphorus (%)	Calcium (%)	Total Energy (Calories per gram)	Total Fat (%)	Total Protein (%)	Total ash (%)	Dry Matter (%)
9.75	0.19	0.47	5059.58	24.40	18.92	5.55	94.86

Table 2: Results of the determination of *S. macrosiphon* cell wall and non-cell wall polysaccharides.

NFC (%)	Total free sugar (%)	Starch (%)	ADL (%)	ADF (%)	NDF (%)	Raw fiber (%)	Hemicellulose (%)	Cellulose (%)	NSP (%)	DF (%)
9.38	4.49	17.25	9.75	23.0	40.50	22.20	17.5	13.26	62.79	53.04

Table 3: Results of tannin content and phenolic compounds of *S. macrosiphon*.

Phenolics (%)	**Tannins (%)**
1.003	0.116

A study on essential oils from 5 medicinal plants, including *Artemisia sieberi, Cymbopogon olivieri, Haplophyllum tuberculatum, Salvia macrosiphon* and *Teucrium polium* suggested that the highest antibacterial activity by the agar diffusion method belonged to *Salvia macrosiphon* [23]. The results of the fatty acid profile analysis of *S. macrosiphon* seeds presented in **Table 4** indicate that the available unsaturated fatty acids are linolenic acid (38.7%), linoleic acid (24.5%), oleic acid (16.7%), palmitoleic acid (1.3%) and saturated fatty acids include palmitic acid (11.2%), myristic acid (0.13%), stearic acid (3.1%), caprylic acid (5.4%). In addition, these seeds lack three fatty acids - capric acid, arachidonic acid, and lauric acid. Similarly, according to studies, as chia seeds contain PUFAs, their popularity and cultivation have greatly expanded recently. Because it contains 64% omega-3 fatty acids and only 19% omega-6 fatty acids, chia seeds are called a "powerhouse of omega fatty acids". Recent research revealed that an imbalanced omega-6(linoleic acid)/omega-3 (alpha-linolenic acid) ratio with a high content of omega-6 PUFAs is strongly proinflammatory and prothrombotic, making it a major contributor to the development of health issues like obesity, diabetes, and atherosclerosis. Therefore, diets with a regular intake of omega-3 PUFAs are linked to decreased cases of these disorders [24, 25].

The results of amino acids analysis of *S. macrosiphon* seed are shown in **Table 5**.

A composition study of *S. macrosiphon* seeds showed that the protein content of SM seeds was 18.92% and that of Chia seeds was 18.21%, and *S. macrosiphon* (SM) seeds closely resembled Chia seeds in terms of protein. The protein content in SM seeds is higher than in grains like oats, barley, rice, and chia seeds; it has great potential in preventing and treating malnutrition. Studies show that regularly consuming protein-rich foods can help lose weight [26]. Furthermore, the content of *S. macrosiphon* seeds is compared with soybean and chia seeds (**Table 6 and 7**).

Table 5: *S. macrosiphon* amino acid analysis results.

Amino acid	Content (%)
Methionine	0.40
Cysteine	0.33
Lysine	0.71
Threonine	0.66
Arginine	1.97
Isoleucine	0.71
Leucine	1.24
Valine	0.89
Histidine	0.49
Phenylalanine	0.91
Glycine	1.06
Serine	1.02
Proline	0.66
Alanine	0.92
Aspartic acid	1.73
Glutamic acid	3.21

Table 4: Results of *S. macrosiphon* fatty acids.

Caprylic acid (%)	Stearic acid (%)	Myristic acid (%)	Palmitic acid (%)	Palmitoleic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
5.40	3.10	0.13	11.20	1.30	16.7	24.5	38.7

The fiber in foods, especially whole grains, is an important factor in maintaining good health due to its beneficial effects, and the paper revealed that the effects of consuming dietary fiber reduce the risk of heart disease, type 2 diabetes, and various types of cancer [26, 27].

Moreover, Appetite reduction has been connected to dietary fiber ingestion. It also improves health by decreasing morbidity. SM seeds have substantially higher fiber content than chia and flax seeds. The prevention of numerous cardiovascular diseases and diabetes has been linked to chia seeds. SM seeds can be used instead of chia seeds to prevent diabetes and cardiovascular disease because their fiber content is substantially higher. **Table 6** shows that the magnesium content of SM plant seeds is higher, while the calcium and phosphorus content of *S. macrosiphon* plant seeds are lower than those of *S. hispanica* plant seeds.

Table 6: Comparison of *S. macrosiphon* and *S. hispanica* seed content.

Content	*S. hispanica*	*S. macrosiphon*
(%)		
Moisture	6.16	5.14
Total ash	4.16	5.55
Oil	34.84	24.34
Crude protein	18.21	18.92
Dietary fiber	23.12	53.04
Linolenic acid	54.0	38.7
Linoleic acid	21.0	24.5
(g/100 g)		
Calcium	0.63	0.47
Phosphorus	0.860	0.186
Magnesium	2.72	9.75

Subsequently, research shows chia seeds are an excellent source of minerals that contain significant amounts of magnesium, calcium, and phosphorus; thus, SM seeds to some extent [28]. SM seeds have an extremely high concentration of polyunsaturated fatty acids. *S. macrosiphon* seeds, like *S. hispanica* seeds, are a rich source of healthy oils because they contain 38.7% linolenic acid and 24.5% linoleic acid. The amino acid profiles of *S. macrosiphon* and *S. hispanica* seeds are compared in **Table 7**. Most of the chia seeds and *S. macrosiphon* seeds have comparable amino acid contents (**Fig. 1**) [26].

Table 7: Comparison of *S. macrosiphon* amino acid profiles with chia and soybean seeds (g/100 g).

Amino acid	Soybeans	Chia	*S. macrosiphon*
Aspartic acid	5.11	1.69	1.73
Threonine	1.77	0.71	0.66
Serine	2.36	1.05	1.02
Glutamic acid	7.88	3.50	3.21
Glycine	1.88	0.9	1.06
Alanine	1.91	1.05	0.92
Valine	2.10	0.95	0.89
Cysteine	0.65	0.41	0.33
Methionine	0.54	0.59	0.40
Isoleucine	1.97	0.80	0.71
Leucine	3.31	1.37	1.24
Lysine	2.71	0.97	0.71
Histidine	1.10	0.53	0.49
Arginine	3.15	2.14	1.97
Proline	2.38	0.77	0.66

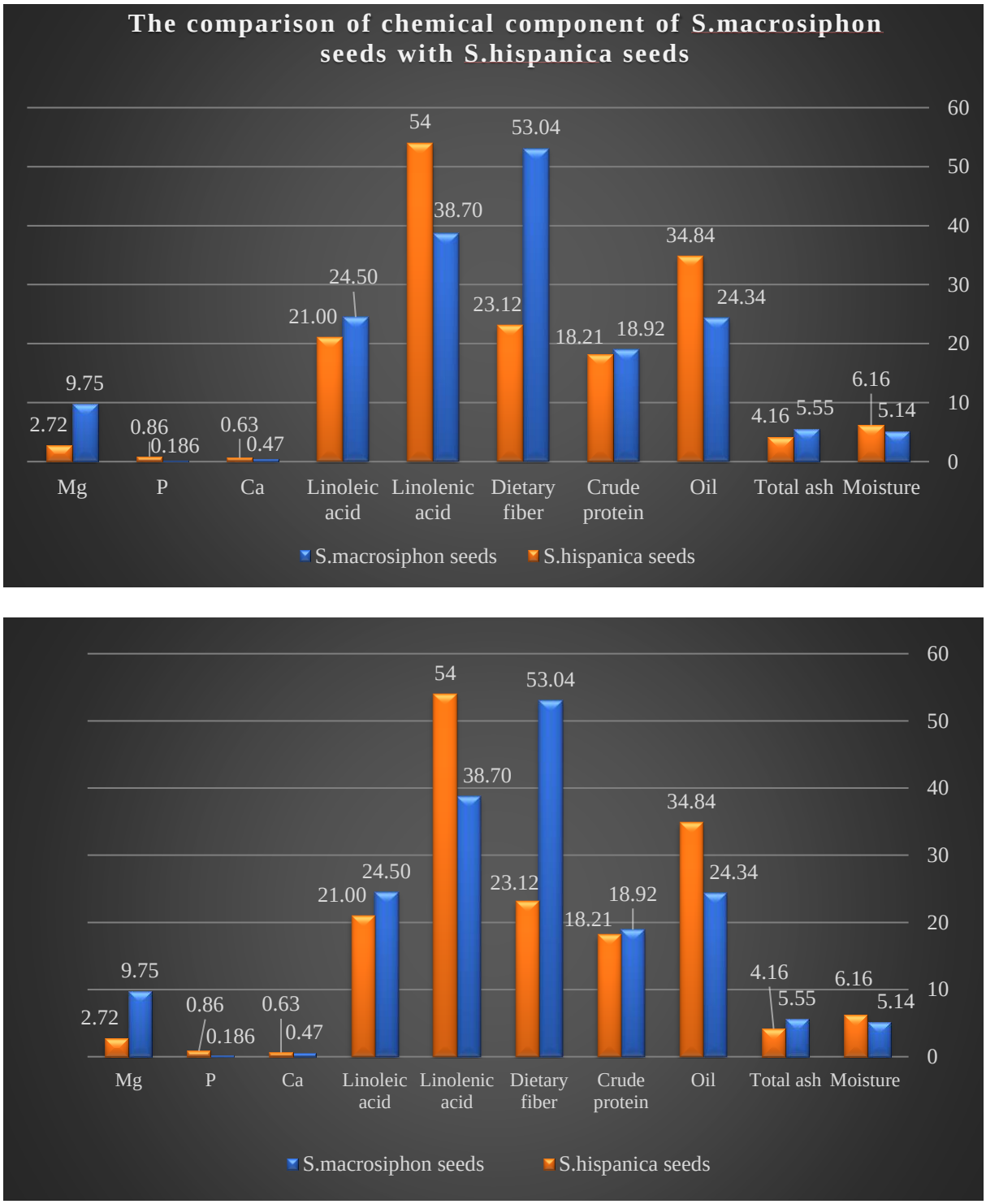

Figure 1. Graph representing proximate analysis of marve seed with the chia seed.

The findings of this study indicate that linolenic and linoleic acid, two polyunsaturated fatty acids, are present in higher concentrations than other fatty acids in the seeds of *S. macrosiphon*. High quantities of unsaturated fatty acids are linked to a lower risk of cancer,

autoimmune diseases, type 2 diabetes, rheumatoid arthritis, hypertension, and coronary artery disease [26, 29]. *S. macrosiphon* seeds may play a significant role in preserving good health due to the physicochemical structure of the cell walls and the high fiber content of these seeds.

According to several studies, eating more fiber lowers the chance of developing cardiovascular disease, type 2 diabetes, and certain types of cancer [27]. Chia seeds provide between 34 and 40 percent of the daily recommended amount of dietary fiber for adults [30]. As a result, chia seeds can significantly contribute to preventing and treating diseases, including diabetes and cardiovascular disease [31]. As *S. macrosiphon* seeds contain considerable dietary fiber (24%), they could play a vital role in human health. Besides, chia seed is an oilseed that contains proteins (15–24%), lipids (40–60% omega-3 fatty acids), dietary fibers (18–30%), carbs (26–41%), and significant amounts of minerals and vitamins, according to another recent study conducting on the chia seeds [32].

4. Conclusion

The analysis of *Salvia macrosiphon* seeds was determined and compared with chia seeds. This work has shown that the seeds contain a notable proportion or weight of substantial components for preventing or treating diseases. Furthermore, similar to the quantity of components obtained from *S. hispanica* seeds, those of *S. macrosiphon* may also have a similar therapeutic effect; nevertheless, it is suggested that additional biological and clinical research be done on *S. macrosiphon* seeds.

Conflict of interest

The authors declare to have no conflict of interest.

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