

## Minia Journal of Agricultural Research and Development

Journal homepage & Available online at:

<https://mjard.journals.ekb.eg>

### Evaluating the Antioxidant Properties of Chia Seed Extract in Enhancing Wound Healing

Yassmina A. El Dabee , M.SH. Hassan, Hemdan I . Mahmoud and Hanaa M. Hassan\*.

Department of Chemistry, Faculty of Agriculture, Minia University, Minia, Egypt.

Received : 25 Nov. 2024

Accepted: 15 Dec. 2024

#### ABSTRACT

Plants have shown considerable significance in phytomedicine, pharmacognosy, and herbal sciences. Chia seeds (*Salvia hispanica*) were sourced from Organic Nation for the examination of their proximity, phytochemical composition (total phenolic and total flavonoid content), and antioxidant activity via the DPPH assay. An examination utilising high-performance liquid chromatography was conducted. The investigation indicates that chia seeds include a high fat content of 24.7%, along with a protein content of 21.05%. The phenolic and flavonoid chemicals detected in the ethanol extract of Chia Seeds, together with the HPLC chromatograms. The predominant phenolic and flavonoid components identified were Kaempferol (340.20 mg/100 g), followed by Rosmarinic acid (9.00 mg/100 g) and chlorogenic acid (1.56 mg/100 g). The primary components of chia oil are polyunsaturated fatty acids (PUFAs), specifically linolenic  $\omega$ -3 fatty acid and linoleic  $\omega$ -6 fatty acid. Nevertheless, the current study suggested utilizing the bioactive compounds found in chia seed mucilage, which are rich in antioxidants, along with omega-3 and omega-6 of fatty acids (linolenic and linoleic) to promote the healing of scratched human cells.

**Keywords:** Chia seed; DPPH; HPLC; PUFA; mucilage.

#### 1. INTRODUCTION

The term "Chia" or "Chien," meaning "oily" in Spanish, denotes an agricultural plant of potential importance (Mohd *et al.*, 2012; Chaudhary *et al.*, 2021). Chia is an exceptional alternative crop for agriculture because to its capacity to thrive in arid circumstances. The significant nutritional benefits have facilitated successful marketing campaigns. Chia is regarded as a nutrient-dense food, comprising substantial

amounts of protein (20%), dietary fiber (20%), carbohydrates (25%), oil (30%), omega-6 polyunsaturated fatty acids (17%), omega-3 polyunsaturated fatty acids (50%), , as well as vitamins, antioxidants, and essential minerals including calcium, manganese, phosphorus, sodium and potassium, chia is classified as a "superfood" or "nutraceutical" due to its health benefits (Prathyusha *et al.*, 2019).

\* Corresponding author: Hanaa M. Hassan

E-mail address: hanaa\_hassan@mu.edu.eg ; hanaa.ammar29@yahoo.com

Chia seeds (*Salvia hispanica* L) are abundant in antioxidants, polyphenols, flavanols, and essential fatty acids, rendering them a significant nutritional asset (Motyka *et al.*, 2023). The antioxidants present in chia seeds significantly contribute to anti-inflammatory processes. The characteristics of chia seeds likely facilitate wound regeneration and repair, so topical chia seed oil has garnered significant attention. Research by Jeong *et al.* (2010) identifies chia seed as an effective topical anti-pruritic treatment for dermatological disorders. Nonetheless, the understanding of the pharmacological properties of chia seed is constrained.

The primary polyphenols in Chia seeds contain caffeic acid (1.75 mg/100 g), catechin (15.47 mg/100 g), cinnamic acid (15.47 mg/100 g), kaempferol (10.51 mg/100 g), p-coumaric acid (2.93 mg/100 g), resveratrol (0.98 mg/100 g), rutin trihydrate (3.98 mg/100 g), syringic acid (0.85 mg/100 g), quercetin (5.01 mg/100 g), and 3,4-dihydroxybenzoic acid (2.30 mg/100 g) (Ghafoor *et al.*, 2022). Diverse qualities including anticoagulant, anti-inflammatory, antioxidant, cardioprotective, hepatoprotective, hypoglycemic, hypolipidemic, hypotensive, and immunostimulatory actions (Ghafoor *et al.*, 2022). Chia seed consists of many bioactive macromolecules, such as oil, protein, and gum, which have shown considerable health advantages in both in vivo and in vitro tests (Chen *et al.*, 2024).

Wound healing is a multifaceted, dynamic process comprising four stages: hemostasis, inflammation, proliferation, and tissue remodeling. During the hemostasis phase, platelet aggregation acts as an initial response to tissue damage, resulting in the formation of a platelet plug, subsequently strengthened by the initiation of fibrin synthesis (Li *et al.*, 2007). In this phase, active platelets secrete many growth factors

and cytokines that significantly affect the subsequent stages of wound healing (Werner and Grose, 2003).

In the inflammatory phase, inflammatory cells such as mast cells, lymphocytes, monocytes, and macrophages invade the damaged tissue. These cells engage in the phagocytosis of bacteria, wound debris, and foreign particles, and are also a crucial source of mediators that promote the proliferative phase (Koh and DiPietro, 2011). In the proliferative phase, various processes transpire, including fibroblast proliferation, collagen production, angiogenesis, re-epithelialization, and wound contraction. Keratinocytes, fibroblasts, and myofibroblasts are the principal cells enabling skin regeneration during this phase (Werner *et al.*, 2007). Ultimately, in the remodeling phase, the wound increases its strength by organizing scar collagen (Rohani and Parks, 2015). Approximately 60-90% of individuals worldwide rely on herbal medicine as their primary form of therapy for numerous common disorders, especially in developing countries. There is a tendency to augment utilization in the future due to the economic efficiency of herbal medicine. Furthermore, individuals exhibit concern regarding pharmacological drugs (WHO, 2002).

The growing importance of herbal medicine in addressing skin disorders and wounds has led to the documentation of plants as treatments for wound healing or dermatological conditions in around 34% of all traditional medicines. In contrast, just 1-3% of modern medications are employed for wound or dermatological therapy (Mantle and colleagues, 2001). In contrast, phytochemicals, acknowledged as advantageous compounds, can be obtained from many plant sources. Their properties facilitate wound healing via multiple mechanisms, including bacterial inhibition, free radical scavenging, and the stimulation

of mitogenic activity, which encompasses promoting cell proliferation, enhancing vascular formation, activating collagen synthesis, and facilitating DNA synthesis (Ghosh and Gaba, 2013). *Salvia hispanica* L., commonly referred to as chia seed, may aid in mitigating inflammatory skin conditions and enhancing wound healing. The pharmacological efficacy and advantages of chia seeds for wound healing require confirmation (Pintapagung and Asawapattanakul, 2020).

This study aims to assess the proximate composition, fatty acid profile, and antioxidant activities of chia seed mucilage, along with its potential role in wound healing.

## MATERIALS AND METHODS

### Plant material.

Chia seeds were sourced from Organic Nation Company in Giza, Egypt. The seeds were ground into a fine powder using an electric grinder (Model MX 491N National).

### Chemicals:

The chemicals utilized in this investigation were acquired from SIGMA-ALDRICH, located at 22 Abo Zar El-Ghafary Street, intersecting El-Tayaran Street, Nasr City, Cairo.

### Proximate Analyses:

The chemical composition of chia seeds, including proteins, lipids, ash, fiber, and carbohydrates, was measured for triplicate samples according to AOAC standard techniques (1990).

### Chia seed mucilage extraction:

Mucilage extraction from chia seeds was conducted with a modified technique derived from the methods outlined by Munoz *et al.* (2012). The seeds were soaked in distilled water at a ratio of 1:3 (weight to volume). The mixture was thereafter agitated continuously at 80°C for 30 minutes. Following extraction, filtration was

conducted using a 250 µm nylon filter, and precipitation was executed with 100% ethanol in a 3:1 volume ratio.

### Quantitative analysis of phytochemicals:

#### Determination the total amounts of phenolics and flavonoids using colorimetric analysis:

The total phenolic content was evaluated via the Folin-Ciocalteu colorimetric technique and reported as gallic acid equivalents (mg Gallic acid/g of extract) (Haq *et al.*, 2012). The total flavonoid content was quantified via aluminium chloride colorimetry and reported as quercetin equivalents (mg of quercetin/g of extract) (Chang *et al.*, 2002).

#### Assessment of antioxidant capacity utilizing DPPH methodology:

The antioxidant properties of Chia seed extract were evaluated by quantifying free radical scavenging activity through the staining of a solvent with the free radical 1,1 diphenyl-2-picrylhydrazyl (DPPH), as outlined by Brand-Williams *et al.* (1995): Two millilitres of chia seed extract at different concentrations (1-64 µg/ml) and a methanol solution as a control were combined with 2 millilitres of DPPH solution (25 mg/L) in methanol. The reaction mixture was agitated vigorously and incubated in darkness for 30 minutes. The absorbance of the combination was measured at 517 nm using a T80 UV/Vis spectrophotometer, using pure methanol as the blank reference. The ratio of radical scavenging activity was evaluated using the following approaches.

**Formula: Radical scavenging (%) = [(A<sub>0</sub> – A<sub>1</sub>) / A<sub>0</sub>] x 100**, where A<sub>0</sub> indicates the absorbance of the control and A<sub>1</sub> signifies the absorbance of the sample extracts. The 50% inhibitory concentration (IC<sub>50</sub>) denotes the concentration required to neutralize 50% of DPPH free radicals.

**Fatty acid analysis by gas chromatography:**

The used of gas chromatography (GC) analysis after the esterification. 0.5 g oil was transferred into 10 mL-capacity glass tube. Five millilitres n-heptane was added into the tube. 200  $\mu$ L 2 M potassium hydroxide solution in methanol was added to this mixture. After mixing for 20 s, upper phase was separated and analysed by GC (TFCC 2010). The GC was equipped with a capillary column (Fused silica, 100 m  $\times$  0.25 mm  $\times$  0.2  $\mu$ m) and a FID detector (Agilent 7890A, Agilent Technologies, USA). The GC conditions used to determine fatty acid methyl ester (FAME) were as follows (TFCC 2010); injection volume: 1 $\mu$ L; temperature programme: 175  $^{\circ}$ C for 10 min, 5  $^{\circ}$ C min $^{-1}$  to 210  $^{\circ}$ C, 5  $^{\circ}$ C min $^{-1}$  to 230  $^{\circ}$ C; final temperature 230  $^{\circ}$ C for 15 min; detector temperature: 260  $^{\circ}$ C; injector temperature: 250  $^{\circ}$ C; gas carrier flow: N<sub>2</sub>, 1 mL min $^{-1}$ ; split: 1:20; total run time: 58.5 min according to Akkaya (2018).

**Techniques of High-Performance Liquid Chromatography:**

An analysis utilizing High-Performance Liquid Chromatography (HPLC) was performed with an Agilent 1260 series apparatus. The separation employed a Zorbax Eclipse Plus C8 column (4.6 mm  $\times$  250 mm i.d., 5  $\mu$ m). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was systematically arranged in a linear gradient as outlined below: 0 min (82% A); 0–1 min (82% A); 1–11 min (75% A); 11–18 min (60% A); 18–22 min (82% A); 22–24 min (82% A). The multi-wavelength detector was evaluated at 280 nm. The injection volume for each sample solution was 5 microliters. The column temperature was maintained at 40  $^{\circ}$ C, as documented by Biswas (2013).

**Cytotoxicity evaluation and Wound healing assay:**

Determination of cytotoxicity on HFB4 cells by using (MTT protocol) according to Ganot *et al.* (2013).

**Protocol for Wound Healing Assay**

Cells were inoculated in six multi-well plates and cultured until confluence was attained. All cultures must converge at the onset of the experiment. A yellow pipette tip was utilized to make a linear incision, mimicking a wound. We generally generate a scratch by angling the pipette tip at roughly 30 degrees to limit the diameter of the scratch. This enables the visualization of both wound edges with the 10x objective.

**Determinations encompass the following items :**

Migration Rate (RM) = (wi – wf) / length

Where: wi represents the average initial wound width in micrometers, Wf denotes the average final wound width in micrometers, and T signifies the assay length in hours.

Wound closure percentage (%) = ((At=0 – At= $\Delta$ t) / At=0)  $\times$  100, where At=0 indicates the initial wound area and At= $\Delta$ t signifies the wound area after n hours .

Percentage change in area = (A<sub>i</sub> - A<sub>f</sub>)

Geographical Position Ai = Initial wound area; Af = Final wound area.

**Statistical evaluation:**

The averages and standard deviations of three parallel measurements made up the experimental data. The analysis of variance was carried out using ANOVA methods. GraphPad Software, San Diego, California, USA, developed GraphPad Prism to calculate statistics at  $P < 0.05$  (Motulsky, 1999).

**RESULT AND DISCUSSION**

**Proximate analysis of Chia seeds:**

The proximate analysis of chia seeds entails assessing their fundamental nutritional constituents, which generally comprise moisture, ash, proteins, fats, carbs, and dietary fiber. Chia seeds (*Salvia hispanica*) are esteemed for their substantial nutritional worth and health advantages, rendering them a favored alimentary source.

Table (1) presents the average values for the chemical composition of chia seeds (% of dry weight). The analysis of chia seed composition revealed elevated levels of fat (24.7%) and protein (21.05%), along with ash (2.7%), fiber (10.04%), and carbohydrates by difference (41.51%). However, the average protein and fat content may vary among different chia seed species based on growth location and environmental conditions.

**Table 1. Proximate analysis of chia seeds (% of dry weight)**

Nutrients (100 g seed dry weight)	chia seeds ( % )
Ash	2.70 ± 0.10
Fiber	10.04 ± 1.7
Protein	21.05 ±0.47
Fat	24.70 ±0.50
carbohydrate	41. 51 ±0.60

Each value is presented as the mean ± standard deviation (n = 3 compositions).

The findings align with those of Suri *et al.* (2016), which indicated that chia seeds contain a protein concentration ranging from 15% to 23%. Coelho and Salas-Mellado (2014), Muñoz-González *et al.* (2019), and Porrás-Loaiza *et al.* (2014) have established that Chia seeds comprise protein (15-25%), fat (15-35%), and ash (4-6%). Chia seeds contain 91 - 93 g of dry matter and 32 - 39 g of oil per 100 g. Chia seeds comprise 4 - 6 g/100g of ash, 22 - 24 g/100g of protein, 18 - 30 g/100g of dietary fiber, and 26 - 41 g/100g of carbohydrates (Guiotto *et al.*, 2011).

**Evaluation of Total Phenolic and Flavonoid Concentrations, as well as Antioxidant Activity of Chia Seeds :**

Table 2 presents the total phenolic content of chia seed mucilage extract, quantified as Gallic acid equivalent, at 20.03

mg/g. Plant phenolics exhibit many biological properties, including antioxidant and anti-inflammatory, anticancer, immune-modulating, cardioprotective, and antibacterial effects (Durazzo *et al.*, 2019). Conversely, Table 2 displayed the overall flavonoid concentration in chia seed mucilage extracts, measured as quercetin equivalents, amounting to 18.15 mg/g. Simultaneously, the antioxidant efficacy of chia seed mucilage extract was assessed utilizing the DPPH method, a stable organic free radical distinguished by its absorption maximum band between 515-528 nm (Stankovi, 2011), often utilized for assessing the antioxidant capacity of various compounds. The results demonstrate that the IC50 of chia seed extract was 54.3µg/ml (Table 2).

**Table (2): Total phenolic compounds , Total flavonoids and DPPH IC<sub>50</sub> of extracts**

Constituent	Total phenolics (mg gallic acid/g) <sup>a</sup>	Total flavonoids (mg quercetin /g) <sup>b</sup>	DPPH IC <sub>50</sub> (µg/ml)*
Chia seed extract	20.03±0.46	18.15±0.26	54.3

a: mg GAE per gram of desiccated leaf extract; b: mg QE per gram of desiccated leaf extract. All values are shown as the mean ± standard deviation (SD).

\*The IC<sub>50</sub> values indicate the quantity of extract necessary to neutralize 50% of the radicals in the reaction mixture.

Kumi *et al.* (2022) established that the total phenolic content of chia seeds ranged from 0.73 to 0.87 mg GAE g<sup>-1</sup>, while the total flavonoid content varied from 0.39 to 0.57 mg GAE g.

#### **-HPLC examination of phenolic and flavonoid constituents in Chia seeds:**

Table 3 outlines the phenolic and flavonoid components identified in the ethanol extract of Chia Seeds, along with the HPLC chromatograms for these substances. The retention periods of the chromatogram peaks were compared with the existing standards, leading to the identification of a restricted number of components (seven). The ethanol extract of Chia Seeds had the highest concentrations of phenolic and flavonoid constituents, with Kaempferol measured at 340.20 mg/100 g, followed by Rosmarinic acid at 9.00 mg/100 g, and chlorogenic acid at 1.56 mg/100 g. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) is a naturally occurring flavonol noted for its various metabolic effects. Cid-Ortega and Monroy-Rivera (2018). Polyphenols possess antioxidant qualities and have been

investigated for their possible health benefits; Rosmarinic acid is a significant constituent of this category, offering many health advantages (Wren and Potter, 1988; Luo *et al.*, 2020). Chlorogenic acid (CGA) is a polyphenolic chemical present in substantial quantities in several plant sources, particularly green coffee beans. CGA, acknowledged as a bioactive natural chemical, demonstrates many therapeutic actions targeting multiple clinical issues, especially those related to chronic metabolic diseases and age-associated disorders (Nguyen *et al.*, 2024). Reyes-Gaudilio *et al.* (2008) shown that chia seeds are a significant source of antioxidants, containing a diverse array of antioxidant chemicals. Many studies have revealed differing concentrations of phenolic chemicals in chia seeds, likely due to variations in analytical and extraction methodologies, temperature, and processing procedures (Ferarsa *et al.* 2018). Therefore, it is essential to implement precautions to prevent the degradation of bioactive components during the thermal processing of chia seeds (Zia-Ud Din *et al.*, 2021).

**Table 3. HPLC analysis of phenolic and flavonoid compounds of Chia seeds:**

Chia seeds	RT (Area)	Conc. (µg/ml)	Biological activity
Chlorogenic acid	11.97	1.56	Antioxidant properties, hepatic and renal protection, antibacterial effects, antitumor activity, control of glucose and lipid metabolism, anti-inflammatory effects, neuroprotection, and vascular action (Wang <i>et al.</i> , 2022)
Coffeic acid	10.98	0.85	Anti-inflammatory and anticancer properties against specific leukemia cell lines (Khan <i>et al.</i> , 2017)
Pyro catechol	2.52	0.36	Utilized for the production of antioxidants, hair dyes, and food additives (Huang <i>et al.</i> , 2014; Suresh <i>et al.</i> , 2012).
Vanillin	8.69	0.32	Possesses multiple applications in food, beverages, medicines, fragrances, and cosmetics (Olatunde <i>et al.</i> , 2022).
Rosmarinic acid	84.08	9.00	Antiviral, antibacterial, anti-inflammatory, antitumor, antioxidant, and antiangiogenic properties (Ijaz <i>et al.</i> , 2023)
Daidzein	3.95	0.22	Pharmacological applications for several illnesses, including oxidative stress, cancer, obesity, cardiovascular problems, neuroprotection, diabetes, ovariectomy, anxiety, and inflammation (Singh <i>et al.</i> , 2023).
Kaempferol	5511.74	340.20	Reducing the risk of chronic diseases such as cancer, liver damage, obesity, and diabetes (Imran <i>et al.</i> , 2019; Wong <i>et al.</i> , 2019). Furthermore, it exhibits anti-inflammatory properties and has been employed in the treatment of several acute and chronic inflammation-related conditions, such as intervertebral disc degeneration, colitis, post-menopausal bone loss, and acute lung injury (Ren <i>et al.</i> , 2019).

**Fatty Acid analysis of chia seed**

Chia seeds comprise 24.70% fat. The fatty acid content of chia oil seeds has been studied via GC-MS, indicating the presence of roughly 13 fatty acids. Table (4) reveals that the principal components of chia oil are polyunsaturated fatty acids (PUFAs),

namely linolenic ω-3 fatty acid and linoleic ω-6 fatty acid. These components comprise up to 58.67% of ω-3 fatty acids and 20.44% of ω-6 fatty acids, yielding a ratio of ω-3 to ω-6 fatty acids of 2.87:1.00. The results align with the findings reported by Luz *et al.* (2012). Conversely, the results indicated that

Chia seeds comprised Myristic acid (0.05%), Palmitic acid (7.98%), and Stearic acid (4.16%) as the predominant saturated fatty acids (Melo *et al*, 2019). Oleic acid,

also known as omega 9, is the predominant monounsaturated fatty acid, comprising approximately 8.15% of chia seed oil.

**Table 4. Gas chromatography analysis of fatty acids in Chia seeds**

Peak	RT	Fatty Acids	Area Sum %
1	21.074	Myristic acid	0.05
2	23.932	Pentadecanoic acid	0.03
3	26.761	Palmitic acid	7.98
4	27.907	Palmitoleic acid	0.06
5	29.457	Margaric acid	0.05
6	32.285	Stearic acid	4.16
7	33.032	Oleic acid	8.15
8	34.827	Linoleic acid	20.44
9	37.1	Linolenic acid	58.67
10	37.922	cis-11-Eicosenoic acid	0.16
11	41.971	Behenic acid	0.1
12	44.26	cis-13,16-Docosadienoic acid	0.03
13	46.488	Lignoceric acid	0.12

RT : Retention Time

#### **Cytotoxicity evaluation and Wound healing assay:**

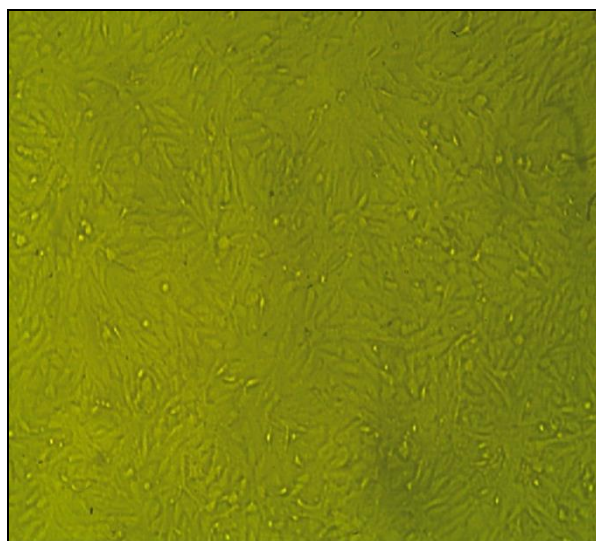
Chia seed extract was reported as mean  $\pm$  standard deviation for three measurements, as illustrated in Table 5.



**Table 5 : Cytotoxicity evaluation using different concentrations 0.625, 1.25, 2.5, 5, 10, 20 µg/ml, of extracts of Chia seed extract against HFB4.**

ID	Conc. µg/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC <sub>50</sub> ± SD
HFB4	-----	0.726	0.729	0.732	0.729	0.001732	100	0	HFB4
Chia seeds	20	0.024	0.027	0.024	0.025	0.001	3.429355281	96.57064472	162.52 ± 1.08
	10	0.063	0.077	0.058	0.066	0.005686	9.053497942	90.94650206	
	5	0.217	0.222	0.219	0.219333	0.001453	30.086877	69.913123	
	2.5	0.465	0.447	0.455	0.455667	0.005207	62.50571559	37.49428441	
	1.25	0.726	0.728	0.73	0.728	0.001155	99.86282579	0.137174211	
	0.625	0.728	0.725	0.733	0.728667	0.002333	99.95427526	0.045724737	

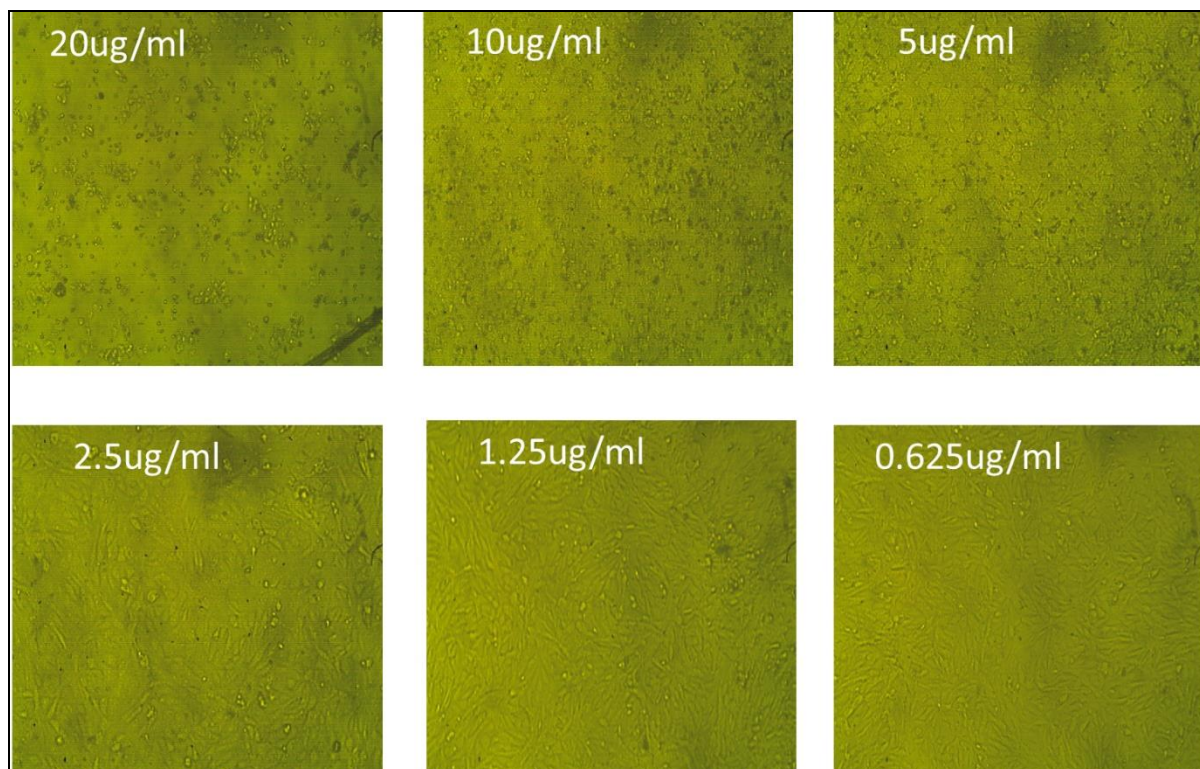
O.D referred to optical density, SE referred to standard error, IC<sub>50</sub> referred to Half maximal inhibitory concentration and calculated mathematically.



**Figure 1: Displays the control HFB4 cells devoid of any influence from Chia seed extract, observed under an inverted microscope with the following specifications: objective lens (10X).**

The control HFB4 cells, which were observed under an inverted microscope using a 10X objective lens (Fig.1.). This figure serves as a baseline for comparison against HFB4 cells treated with Chia seed extract. The control HFB4 cells appear healthy, exhibiting normal morphology characteristic of this cell line. This includes

consistent cell shape and size, which is important for establishing a reliable baseline. This figure sets a foundation for comparing treated cells in subsequent figures. Observations of any significant deviations in treated samples will be crucial for assessing the effects of Chia seed extract.



**Figure 2: Demonstrates the impact of Chia seed extract on HFB4 cells at varying doses.**

**Wound healing Assay: scratch wound healing assay**

The human skin is consistently exposed to both extrinsic and intrinsic natural factors, such as environmental pollution, photoaging, and chronological aging, which lead to skin degradation. The main changes associated with skin degeneration in appearance include coarse texture, blemishes, wrinkles, and a notable loss of elasticity. The cosmetic industry has shown

a keen interest in innovative formulations of skin cosmetics that incorporate bioactive chemicals offering both preventative and therapeutic benefits (Lorecini *et al.*, 2014; Ramos-e-Silva, 2013). The skin can experience abrasion or injury and may be treated with chemicals or cosmetics for protection.

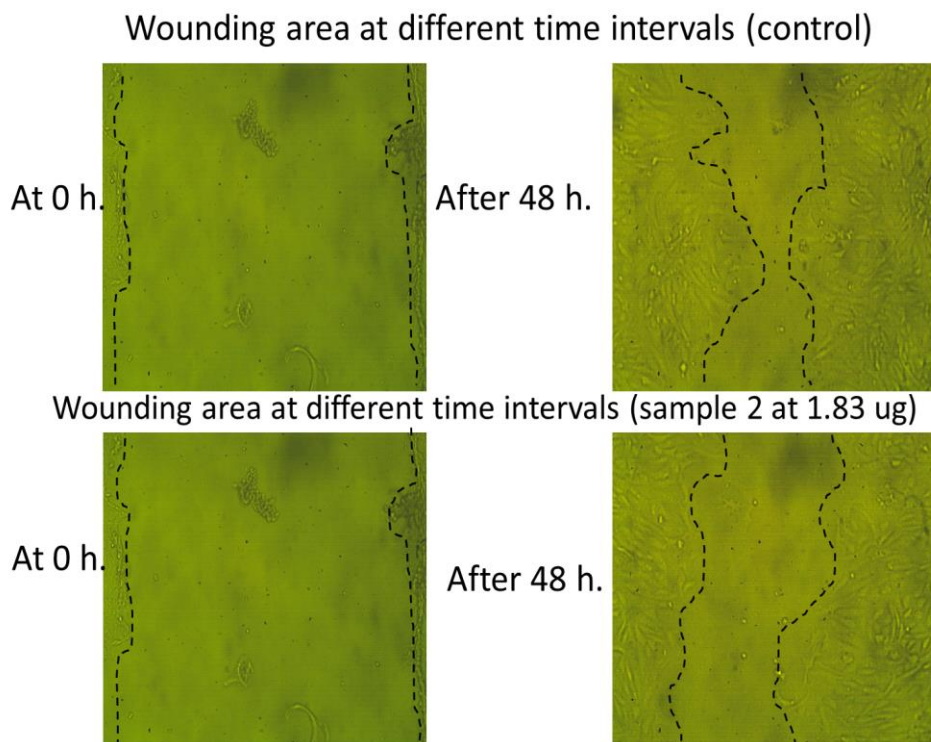
The present study supports the use of bioactive compounds derived from chia seed mucilage, known for their high antioxidant

content and omega-3 and omega 6 fatty acids (linolenic and linoleic), to enhance the healing process of scratched human cells and reduce oxidative stress in damaged cells. The data presented in Table 6 and illustrated in Figure 3 demonstrate that the use of chia seed mucilage on the damaged cells reduced the time needed to decrease wound width (from 13.44391  $\mu\text{m}$  in the

control group to 12.43036  $\mu\text{m}$ ).The treatment resulted in a reduction of the wound area or percentage of wound closure, decreasing from 69.00617  $\mu\text{m}^2$  in the control group to 63.8183  $\mu\text{m}^2$ . Additionally, the area difference percentage was reduced from 489973.7% in the control group to 453137.6%.

**Table (6): Wounding area at different time intervals ( hour ) .**

Item	At zero hour		Compounds		RM $\mu\text{m}$	Wound closure % $\mu\text{m}^2$	Area difference
	width	area	width	Area			
<b>Control wounded cells</b>	935.0193	710043.3	289.7118	220069.6	13.44391	69.00617	489973.7
<b>Heal wounded cells of Chia seed</b>	935.0193	710043.3	338.362	256905.7	12.43036	63.8183	453137.6



**Figure (3) Wounding area at different time intervals (Control and heal wounded of Chia seed extract: sample 2).**

## CONCLUSION

The current findings indicate that chia seeds contain substantial amounts of fibre, lipids (including omega-3 and omega-6 fatty acids), and protein, while their mucilage extracts demonstrate notable antioxidant capabilities. The high levels of bioactive phenolic compounds, flavonoids, and antioxidant activity, along with the bioactive components of chia seed mucilage, which is abundant in antioxidants and omega-3 and omega-6 fatty acids (linolenic and linoleic acids), promote the repair of damaged human cells.

## REFERENCES

- Akkaya, M.R. (2018).** Prediction of fatty acid composition of sunflower seeds by near-infrared reflectance spectroscopy. *J Food Sci Technol* ;55(6):2318-2325.
- Bae, J., Kim, N. and Shin, Y. (2020).** Activity of catechins and their applications. *biomed dermatol* 4, 81
- Boly, R., Lamkani, T., Lompo, M., Dubois, J. and Guissou I (2016).**DPPH Free Radical Scavenging activity of two extracts from *Agelanthus dodoneifolius* (Loranthaceae) leaves. *Int J Toxicol Pharmacol Res* 8(1):29–34
- Chang, C. C., Yang, M. H., Wen, H. M. & Chern, J. C. (2002).** Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10, 178–182.
- Chaudhary, N.; Dangi, P.; Kumar, R. and Bishnoi, S. (2021).** ( Chia Seeds—A Renewable Source as a Functional Food. *Handbook of Cereals, Pulses,*

*Roots, and Tubers*, CRC Press, Boca Raton, 2021, pp. 235–252.

- Chen J.; Wu G., Zhu L.; Karrar E.; and Zhang H.(2024)** A review of the functional activities of chia seed and the mechanisms of action related to molecular targets, *Food Funct.*, 2024,**15**, 1158-1169.
- Cid-Ortega S and Monroy-Rivera JA. (2018).** Extraction of kaempferol and its glycosides using supercritical fluids from plant sources: A review. *Food Technol Biotechnol.* ;56:480–493.
- Coelho MS, Salas-Mellado M (2014).** Chemical characterization of chia (*Salvia hispanica* L.) for use in food products. *Journal of Food and Nutrition Research* 2(5):263-269.
- Ghafoor, K.; AlJuhaimi, F.;Ozcan, M.M.; Uslu, N.; Ahmed, I.A.M. and Babiker, E.E. (2022).** The effect of boiling, germination and roasting on bioactive properties, phenolic compounds, fatty acids and minerals of chia seed (*Salvia hispanica* L.) and oils, *Int J Gastron Food Sci* 27 , 100447.
- Ghosh, P.K., Gaba, A., (2013).** Phyto-extracts in wound healing. *J. Pharm. Pharm. Sci.* 16, 760-820.
- Guiotto, E.N., Ixtaina, V.Y., Tomás, M.C. and Nolasco, S.M. (2011)** Moisture Dependent Physical Properties of Chia (*Salvia hispanica* L.) Seeds. *American Society of Agricultural and Biological Engineers*, 54, 527-533.
- Haq, I. (2012).**Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iran J. Pharm. Res.* 11, 241–249.

- Huang, S., Zhang, C.-P., Li, G. Q., Sun, Y.-Y., Wang, K., and Hu, F.-L. (2014).** Identification of catechol as a new marker for detecting propolis adulteration. *Molecules*, 19(7), 10208-10217.
- Ijaz, S.; Iqba, J.; Abbasi, B. A.; Ullah, Z.; Yaseen, Kanwal, T.; Mahmood, S.T.; Sydykbayeva, S.; Ydyrys, A.; Almarhoon, Z. M. Sharifi-Rad, J.; Hano, C.; Calina, D.; Cho, W. C.; (2023)** .Rosmarinic acid and its derivatives: Current insights on anticancer potential and other biomedical applications ., *Biomedicine & Pharmacotherapy*, 162,114678.
- Imran M, Rauf A, Shah ZA, Saeed F, Imran A, Arshad MU, (2019).** Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: A comprehensive review. *Phytother Res.*;33:263–275.
- Kahkeshani N, Farzaei F, Fotouhi M, Alavi S.S, Bahramsoltani R, Naseri R, Momtaz S, Abbasabadi Z, Rahimi R, Farzaei M.H. and Bishayee A. (2019).** Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iran J Basic Med Sci.* 22(3):225-237.
- Khan F.A., Maalik A. and Murtaza G. (2017).**Inhibitory mechanism against oxidative stress of caffeic acid, *J. Food Drug Anal.* 24 (4) (2017) 695–702.
- Koh, T.J., and DiPietro, L.A., (2011).** Inflammation and wound healing: the role of the macrophage. *Expert Rev. Mol. Med.*13.
- Li, B. and Yang, X. (2020). Rutin-loaded cellulose acetate/poly (ethylene oxide) fiber membrane fabricated by electrospinning: a bioactive material. *Materials Science and Engineering: C*, 109, 110601.
- Li, J., Chen, J., Kirsner, R., 2007. Pathophysiology of acute wound healing. *Clin. Dermatol.* 25, 9-18.
- Luo C., Zou L., Sun H., Peng J., Gao C., Bao L., Ji R., Jin Y. and Sun S. A.(2020).** Review of the Anti-Inflammatory Effects of Rosmarinic Acid on Inflammatory Diseases. *Front.Pharmacol.* ;11:15
- Mantle, D., Gok, M.A. and Lennard, T.W., (2001).** Adverse and beneficial effects of plant extracts on skin and skin disorders. *Adverse Drug React. Toxicol. Rev.* 20, 89-103.
- Mohd, A. N.; Yeap, S.K., Ho, W.Y.; Beh, B.K.; Tan, S.W and Tan, S.G. (2012).**The promising future of chia, *Salvia hispanica L*, *J Biomed Biotechnol* 2012 .
- Motulsky, H. (1999).** Analyzing Data with GraphPad Prism, GraphPad Software Inc. San Diego, CA, 92121.
- Motyka, S.; Skąła, E.; Ekiert, H. and Szopa, A.(2023).** Health-promoting approaches of the use of chia seeds, *J Funct Foods* 103 (2023) 105480, <https://doi.org/10.1016/J.JFF.2023.105480>.
- Munoz, L.A., Aguilera, J.M., Rodriguez-Turienzo, L., Cobos, A., Diaz, O., (2012).** Characterization and microstructure of films made from mucilage of *Salvia hispanica* and whey protein concentrate. *J. Food Eng.* 111 (3), 511–518.
- Muñoz-González I, Merino-Álvarez E, Salvador M, Pintado T, RuizCapillas C, Jiménez-**

- Colmenero F. and Herrero AM (2019).** Chia (*Salvia hispanica L.*) a Promising Alternative for Conventional and Gelled Emulsions: Technological and Lipid Structural Characteristics 5:19
- Nguyen, V.; Taine, E.G.; Meng, D.; Cui, T. and Tan, W. Chlorogenic Acid: A Systematic Review on the Biological Functions, Mechanistic Actions, and Therapeutic Potentials. *Nutrients* 2024, *16*, 924.
- Olatunde, A., Mohammed, A., Ibrahim, M. A., Tajuddeen, N. and Shuaibu, M. N. (2022).** Vanillin: A food additive with multiple biological activities, *European Journal of Medicinal Chemistry Reports*, Volume 5,100055.
- Pintapagung T. and Asawapattanakul T. (2020).** Effect of chia (*Salvia hispanica L.*) seed extract on wound healing in mice. *Veterinary Integrative Sciences*; *18*(2): 103-117.
- Porras-Loaiza P, Jiménez-Munguía M.T, Sosa-Morales M.E, Palou E., López- and Malo A. (2014).** Physical properties, chemical characterization and fatty acid composition of Mexican chia (*Salvia hispanica L.*) seeds. *International Journal of Food Science and Technology* *49*:571-577.
- Prathyusha, P.; Kumari, B.A.;Suneetha, W.J. and Sai, M.N. (2019).** Chia seeds for nutritional security, *J Pharmacogn Phytochem* *8*, 2702–2707.
- Ren, J., Lu, Y., Qian, Y., Chen, B., Wu, T. and Ji G. (2019).** Recent progress regarding kaempferol for the treatment of various diseases. *Exp Ther Med.* ;*18*:2759–2776.
- Rohani, M.G. and Parks, W.C., (2015).** Matrix remodeling by MMPs during wound repair. *Matrix Biol.* *44-46*, 113-121.
- Shimsa S., Mondal S. and Mini S. (2024).** Syringic Acid: A Promising Phenolic Phytochemical with Extensive Therapeutic Applications. *R&D of Functional Food Products*; *1*(5): 1-14.
- Singh S., Grewal S., Sharma N., Behl T., Gupta S., Anwer M.K. and Vargas-De-La-Cruz C., Mohan, S. and Bungau SG, Bumbu A. (2023).** Unveiling the Pharmacological and Nanotechnological Facets of Daidzein: Present State-of-the-Art and Future Perspectives. *Molecules.* *13*;28(4):1765.
- Suresh, S., Srivastava,V. C., and Mishra, I. M. (2012).** Adsorption of catechol, resorcinol, hydroquinone, and their derivatives: a review. *International Journal of Energy and Environmental Engineering*,*3*,1-19.
- Suri, S., Passi, S.J., and Goyat, J. (2016).** Chia seed (*Salvia hispanica L.*)—A new age functional food. In: *4th International Conference on Recent Innovations in Science Engineering and Management* pp. 286-299.
- Wang, L., Pan, X., Jiang, L., Chu, Y., Gao, S., Jiang, X., Zhang, Y., Chen, Y., Luo, S. and Peng C. (2022).** The Biological Activity Mechanism of Chlorogenic Acid and Its Applications in Food Industry: A Review. *Front Nutr.* *29*;9:943911.
- Werner, S. and Grose, R., (2003).** Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* *83*, 835-870.

**Werner, S., Krieg, T. and Smola, H., (2007).** Keratinocyte-fibroblast interactions in wound healing. *J. Invest. Dermatol.* 127, 998-1008.

**WHO, (2002).** WHO Traditional Medicine Strategy 2002–2005. WHO Geneva, pp. 7-15.

**Wong, S.K., Chin, K.Y. and Ima-Nirwana, S. (2019).** The osteoprotective effects of kaempferol: the evidence from in vivo and in vitro studies. *Drug Des Devel Ther.* ;13:3497–3414.

**Wren R. and Potter S.(1988).***New Cyclopaedia of Botanical Drugs and Preparations.* The CW Daniel Co. Ltd.; London, UK:

**Zhang, W., Chen, H., Wang, Z., Lan, G., & Zhang, L. (2013).** Comparative studies on antioxidant activities of extracts and fractions from the leaves and stem of *Epimedium koreanum* Nakai. *Journal of food science and technology*, 50, 1122-1129.

## تقييم خصائص مستخلص بذور الشيا المضادة للأكسدة في تعزيز التنام الجروح

ياسمينا أحمد الضبع ، محمد شعبان حسن ، حمدان إبراهيم محمود ، هناء محمد حسن  
قسم الكيمياء الزراعية . كلية الزراعة . جامعة المنيا

أثبتت النباتات أهمية كبيرة في الطب النباتي وعلم العقاقير وعلوم الأعشاب. تم شراء بذور الشيا (*Salvia hispanica*) من شركة Organic Nation حتى يمكن تحليلها لمعرفة مدى محتواها الكيميائي النباتي (إجمالي الفينول وإجمالي محتوى الفلافونويد) ونشاط مضادات الأكسدة باستخدام DPPH. بالإضافة إلى ذلك، تم إجراء تحليل كروماتوغرافيا سائلة عالية الأداء. يشير التحليل إلى أن تركيبة بذور الشيا تحتوي على كميات أعلى من كل من الدهون (٢٤.٧٪) تليها البروتين (٢١.٠٥٪). المواد الكيميائية الفينولية والفلافونويدية التي تم تحديدها في مستخلص الإيثانول من بذور الشيا، جنباً إلى جنب مع كروماتوغرافيا HPLC. كانت المكونات الفينولية والفلافونويدية الأكثر انتشاراً هي كامفيرول (٣٤٠.٢٠ مجم / ١٠٠ جم) ثم حمض روزمارينيك (٩.٠٠ مجم / ١٠٠ جم) وحمض الكلوروجينيك (١.٥٦ مجم / ١٠٠ جم). المكونات الرئيسية لزيت الشيا هي الأحماض الدهنية المتعددة غير المشبعة (PFAs) أوميغا ٣ (حمض اللينولينيك) وأميغا ٦ (حمض اللينولييك). ومع ذلك، اقترحت الدراسة الحالية استخدام المركبات النشطة بيولوجياً في مخاط بذور الشيا ذات مضادات الأكسدة العالية جنباً إلى جنب مع محتويات أحماض أوميغا ٣ وأوميغا ٦ (أحماض الدهنية اللينولينيك واللينولييك) لعلاج الخلايا البشرية المخدوشة.