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Comparative evaluation of physicochemical properties, fatty acid profile, anticholinesterase and anti-inflammatory activities of cold pressed chia, linseed, and rapeseed oils

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SUMMARY: Cold-pressed oils from chia (*Salvia hispanica* L.), flax (*Linum u sitatissimum* L .), a nd r apeseed (*Brassica n apus* L .) were evaluated for physicochemical properties, fatty acid composition, and in vitro biological activities. Chia oil showed the highest saponification value and the lowest peroxide and acid values, indicating superior oxidative quality. GC-FID analysis revealed high αα-linolenic acid levels in chia and flaxseed oils (63.3 and 56.25%), whereas rapeseed oil was rich in oleic acid (64.35%). Chia oil exhibited the strongest anti-inflammatory activity (IC50=2.46 mg/mL) (*IC*50=2.46 mg/mL). Chia and flaxseed oils also showed stronger acetylcholinesterase and butyrylcholinesterase inhibitory effects than rapeseed oil. These findings suggest that αα-linolenic acid-rich oils, particularly chia oil, possess promising nutritional and bioactive potential, while rapeseed oil may offer better oxidative stability and culinary value.

KEY-WORDS: *Cholinesterase inhibitory activity; Chromatographic analysis; Extraction; Vegetable oils*.

RESUMEN: ***Evaluación comparativa de las propiedades fisicoquímicas, el perfil de ácidos grasos, la actividad anticolinesterásica y antiinflamatoria de los aceites de chía, linaza y colza prensados en frío.*** Se evaluaron las propiedades fisicoquímicas, la composición de ácidos grasos y las actividades biológicas in vitro de aceites prensados en frío de chía (Salvia hispanica L.), lino (Linum usitatissimum L.) y colza (Brassica napus L.). El aceite de chía presentó el mayor índice de saponificación y los menores valores de peróxido y acidez, lo que indica una calidad oxidativa superior. El análisis GC-FID reveló altos niveles de ácido α-linolénico en los aceites de chía y lino (63,3 % y 56,25 %, respectivamente), mientras que el aceite de colza fue rico en ácido oleico (64,35 %). El aceite de chía mostró la mayor actividad antiinflamatoria (CI50 = 2,46 mg/mL). Los aceites de chía y lino también mostraron mayores efectos inhibidores de la acetilcolinesterasa y la butirilcolinesterasa que el aceite de colza. Estos hallazgos sugieren que los aceites ricos en ácido αα-linolénico, en particular el aceite de chía, poseen un potencial nutricional y bioactivo prometedor, mientras que el aceite de colza podría ofrecer una mayor estabilidad oxidativa y valor culinario.

PALABRAS CLAVE: *Aceites vegetales; Actividad inhibidora de la colinesterasa; Análisis cromatográfico; Extracción.*

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1. INTRODUCTION

Vegetable oils have garnered increasing attention due to their nutritional value and associated health benefits, particularly those extracted from seeds such as chia (*Salvia hispanica* L.), linseed (*Linum usitatissimum* L.), and rapeseed (*Brassica napus* L.). These oils are rich in essential fatty acids, antioxidants, vitamins, and phytochemicals, making them important functional foods in the prevention and management of chronic diseases, especially cardiovascular and metabolic disorders (Ullah *et al.,* 2016).

Chia seeds, native to Central and South America, especially Mexico and Guatemala, have been cultivated for centuries as a staple crop. They are a rich source of alpha-linolenic acid (ALA), dietary fiber, antioxidants, and plant-based protein, contributing to cardiovascular protection, improved digestion, and glycemic regulation (Ullah *et al.*, 2016).

Linseed, also known as flaxseed, originates from the Mediterranean region and is now widely cultivated in temperate countries such as Canada, Russia, and parts of Europe. It is one of the richest plant sources of omega-3 fatty acids and lignan compounds with antioxidant and phytoestrogenic activities which have been associated with reduced risk of cardiovascular disease, certain cancers, and diabetes (Al-Madhagy *et al.,* 2023).

Rapeseed oil, extracted from *Brassica napus* L., is a widely cultivated oilseed crop in Canada, India, China, and several European countries. It is characterized by a favorable fatty acid profile, particularly high levels of monounsaturated fats and omega-3 fatty acids, which contribute to reducing low-density lipoprotein (LDL) cholesterol and supporting cardiovascular health. Moreover, it contains vitamin E and phytosterols which enhance its antioxidant and anti-inflammatory potential (Raboanatahiry *et al.,* 2021).

To extract these valuable oils, various techniques are employed, including mechanical pressing, solvent extraction, and supercritical fluid extraction. Among them, cold pressing, a mechanical method conducted at low temperatures, is widely preferred for producing high-quality oils. This method preserves sensitive bioactive compounds and nutrients that might be degraded under high heat or chemical processing, while also maintaining the natural flavor and aroma of the seeds (Vukic and Vujasinovic, 2023).

In recent years, oilseeds such as chia, linseed, and rapeseed have attracted considerable scientific interest due to their potential health-promoting properties and benefits for human wellness, including anti-inflammatory, cardioprotective, and antioxidant effects. Most studies have focused on the fatty acid composition, vitamin and phenolic contents of oils extracted using different techniques, as well as their oxidative stability under various storage conditions. More recently, increasing attention has been given to the biological activities of these compounds and their role in promoting human health and preventing chronic diseases. However, to the best of our knowledge, the anticholinesterase and anti-inflammatory activities of these oils have not yet been thoroughly investigated. Therefore, to address this gap, the present study aims to provide a comprehensive comparative evaluation of the fatty acid profile, physicochemical characteristics, anticholinesterase (ChE), and in vitro anti-inflammatory properties of cold-pressed chia, linseed, and rapeseed oils produced in Algeria.

2. MATERIALS AND METHODS

2.1. Seed origin, preparation and cold-pressed oil extraction

Chia (*Salvia hispanica* L.), linseed (*Linum usitatissimum* L.), and rapeseed (*Brassica napus* L.) seeds were purchased from a local supplier. Comprehensive details on the geographic provenance, varietal identification, and storage protocols are provided in Table 1. Upon arrival, the seeds were manually cleaned to remove dust, debris, and broken or damaged specimens. They were then sterilized by rinsing with a dilute ethanol solution (70%) and air-dried at room temperature under sterile conditions. For each seed type, 1 kg of clean seeds was used for oil extraction. The seeds were subjected to mechanical cold pressing using a screw-type cold-press machine, with the temperature continuously monitored and maintained within the range of 32–38 °C to preserve thermolabile compounds and ensure oil quality. No solvents or additives

were used during extraction. The process was conducted under aseptic conditions to prevent microbial contamination. The crude oils were immediately collected in pre-sterilized, hermetically sealed dark glass bottles. They were left undisturbed for 15 days at room temperature (22–25 °C) to allow the natural sedimentation of suspended solids. After this settling period, the supernatant oils were carefully decanted and filtered through sterile mesh filters (0.45 µm pore size) to remove residual particulates. The filtered oils were stored in opaque, tightly sealed amber glass bottles at 4 °C until further analysis.

TABLE 1. Specification of plant materials: geographic origin, varietal information, and technical certification standards

Seed	Local Name	Variety	Geographic origin	Coordinates	Certification & Authority	Post-Harvest Storage
Linseed	Zeriâet El Kettane	Lirina	Sétif (local)	36.19° N, 5.41° E	Certified (CNCC National Catalog)	Moisture: 9–10.5%. Temp: 15–25°C. Ventilated silos.
Rapeseed	Colza	Zitna / Baraka	Constantine (local)	36.35° N, 6.60° E	Certified (CNCC / ITGC Priority Program)	Moisture: 7–9%. Highly sensitive; requires rapid drying.
Black Chia	Chia Sawda	Conventional Black	**Paraguay** (Import)	25.30° S, 57.62° W	**Phytosanitary Cert** (SENAVE Py / Algerian Customs)	Moisture: 8% Max. Store in 25kg PP bags.

2.2. Physicochemical characteristics of the oils

2.2.1. Acidity index (AI)

The acidity index (AI) of the oil samples was determined in accordance with the French standard NF T 60-204 (AFNOR), which is based on the titration of free fatty acids with a standardized base.

Precisely 10 g of each oil sample were weighed into a clean, dry 250-mL conical flask. The sample was dissolved in 100 mL of neutralized 95% ethanol, and the mixture was gently heated to near its boiling point (~70–80 °C) to ensure complete dissolution of the oil. While the solution was still warm, titration was carried out using a 0.1 N potassium hydroxide (KOH) solution with phenolphthalein as a visual indicator. The titration was performed with continuous swirling until a stable pale pink endpoint persisted for at least 30 seconds, indicating complete neutralization of the free fatty acids present in the sample.

2.2.2. Peroxide index (PI)

The peroxide index (PI) was determined following the standardized method NF T 60-220 (AFNOR). This method is based on the ability of lipid peroxides to oxidize potassium iodide (KI), resulting in the release of iodine (I_2) in an acidic medium. The amount of iodine liberated is stoichiometrically equivalent to the peroxide content in the oil and is titrated to quantify the extent of primary lipid oxidation. Briefly, 1.00 gram of the oil sample was accurately weighed and placed into a conical flask. To this, a mixture of chloroform and glacial acetic acid (2:3 v/v) was added to dissolve the sample. Then, 1 mL of a saturated potassium iodide (KI) solution was added to the mixture. The flask was closed immediately and allowed to stand in the dark at room temperature for 5 minutes to ensure complete reaction. After this period, 75 mL of distilled water were added, and the liberated iodine was titrated with a 0.01 N sodium thiosulfate ($Na_2S_2O_3$) solution until the yellow color faded. A few drops of freshly prepared starch indicator were then added, which turned the solution blue. Titration continued until the blue color disappeared, indicating the endpoint.

2.2.3. Saponification value (SV)

The saponification value (SV) was determined according to the procedure described by Halaldeen *et al.* (2024), which measures the amount of potassium hydroxide (KOH) required to saponify one gram of oil. Precisely 1.00 gram of the oil sample was weighed into a clean, dry conical flask. Then, 15 mL of a 1 N alcoholic

potassium hydroxide (KOH) solution and 10 mL of distilled water were added. The mixture was refluxed under a water-cooled condenser for 30–40 minutes to ensure complete saponification of the triglycerides.

After cooling to room temperature, a few drops of phenolphthalein indicator were added. The excess KOH was then titrated with 0.5 M hydrochloric acid (HCl) until the pink coloration disappeared, indicating the endpoint. A blank determination (without oil) was carried out simultaneously under identical conditions to correct for any reagent impurities.

2.2.4. Ester index (EI)

The ester index (EI) is a calculated parameter that represents the difference between the saponification value (SV) and the acidity index (AI) of an oil sample. It provides an estimate of the esterified portion of fatty acids (i.e., triglycerides), by indicating the amount of potassium hydroxide (KOH) required to saponify the ester bonds in one gram of fatty material, excluding the free fatty acids. The ester index was calculated using the following equation:

$$EI = SV - AI$$

Where:

SV is the saponification value.
AI is the acidity index.

A higher ester index typically reflects a higher content of triglycerides and a lower proportion of free fatty acids, suggesting a fresher, less degraded oil. Conversely, a lower ester index may indicate a hydrolytic degradation of triglycerides due to oxidation, enzymatic activity, or improper storage conditions.

2.2.5. Density

The density of the oil samples was determined using a pycnometric method in accordance with the AFNOR standard. This method is based on comparing the mass of an equal volume of oil and distilled water at a controlled temperature.

A clean and dry pycnometer was first rinsed with ethanol followed by acetone, and then dried thoroughly. The empty pycnometer was weighed to obtain its tare mass (m_0). Next, it was filled with distilled water, and the sample was thermostated in a water bath at 20 °C for 30 minutes to ensure temperature equilibration. The pycnometer was then weighed again to record the mass with water (m_1). The procedure was repeated with the oil samples: the pycnometer was filled with approximately 2 grams of oil, equilibrated at 20 °C for 30 minutes, and weighed to determine the mass with oil (m_2). The density (ρ) of the oil was calculated using the following formula:

$$\rho = (m_2 - m_0) / (m_1 - m_0)$$

Where:
m_0 = mass of the empty pycnometer (g)
m_1 = mass of the pycnometer filled with water (g)
m_2 = mass of the pycnometer filled with oil (g)

The result is expressed in grams per cubic centimeter (g/cm³). Oil density is an important physical property that correlates with molecular weight and degree of unsaturation of the fatty acids present.

2.2.6. Refractive Index (RI)

The refractive index (RI) of the oil samples was determined using a digital refractometer, following the procedure described by Hu *et al.,* (2024). The instrument was calibrated with distilled water at 20 °C to ensure accuracy.

Approximately 1–2 mL of each oil sample were carefully placed on the clean, dry prism surface of the refractometer. Measurements were carried out at a controlled temperature of 20 °C, and the RI values were recorded directly from the instrument.

The refractive index is a dimensionless parameter that reflects the extent to which light is bent (refracted) when passing through the oil. It is influenced by the degree of unsaturation, chain length, and presence of impurities in the fatty acids. Thus, RI serves as a useful indicator of oil purity, composition, and potential adulteration.

2.3. GC-FID Analysis of oil's fatty acids

The fatty acid composition of the oil samples was determined using gas chromatography with a flame ionization detector (GC-FID), following the method described by Khan *et al.* (2024). Prior to analysis, the oil samples were subjected to transesterification to convert the triglycerides into fatty acid methyl esters (FAMEs), which are more volatile and suitable for gas chromatography. Briefly, 50 mg of each oil sample were dissolved in *n*-hexane, and 2 mL of 2 M potassium hydroxide (KOH) in methanol were added. The mixture was vigorously shaken and heated at 60 °C for 15 minutes to facilitate the methylation (transesterification) reaction. After cooling, the completeness of transesterification was verified by thin-layer chromatography (TLC) using silica gel plates with hexane:diethyl ether (80:20, v/v) as the mobile phase. The absence of a triglyceride spot confirmed complete conversion. After phase separation, the upper FAME-containing layer was carefully collected, further diluted with *n*-hexane, and transferred into a GC vial for analysis.

The analysis was performed on an Agilent 7820A gas chromatograph equipped with a flame ionization detector (FID) and a capillary column (SP-2560, 100 m × 0.25 mm i.d., 0.2 μm film thickness). The injection volume was 1 μL, with a split ratio of 20:1. Helium was used as the carrier gas, while hydrogen and dry air were used for the FID, at flow rates of 40 and 450 mL/min, respectively. The injector and detector temperatures were set at 250 and 260 °C, respectively. The oven temperature was initially held at 100 °C for 5 minutes, then increased to 240 °C at a rate of 4 °C/min, and held at this final temperature for 20 minutes. Fatty acid methyl esters were identified by comparing their retention times with those of certified FAME standards. The quantification was based on the peak area percentages calculated from the FID response.

2.4. Anti-inflammatory activity

The *in vitro* anti-inflammatory activity of the oil samples was evaluated using the protein denaturation assay, as described by Kellab *et al.* (2024). Briefly, 0.5 mL of a bovine serum albumin (BSA) solution (0.2% w/v prepared in 0.05 M tris-buffered saline, pH 6.6) was mixed with 0.5 mL of oil at various concentrations (2000, 1000, 500, and 250 μg/mL). The mixtures were incubated at 37 °C for 15 minutes in an oven, followed by heating in a water bath at 70 °C for 5 minutes to induce protein denaturation. After cooling to room temperature, the turbidity of each solution was measured spectrophotometrically at 660 nm, which correlates with the degree of protein precipitation. A control solution consisted of BSA mixed with 50 μL of methanol instead of oil. Diclofenac sodium was used as the reference anti-inflammatory standard.

The percentage inhibition of protein denaturation (% inhibition) was calculated using the formula:

$$\text{Inhibition } \% = (1 - At/Ac) \times 100$$

where:

At is the absorbance of the test sample.

Ac is the absorbance of the control.

2.5. Cholinesterase (BChE and AChE) inhibitory activity

Butyrlcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities were determined based on the method previously described by Elkolli *et al.* (2022) with slight modifications. 150 μL of

sodium phosphate buffer (100mM, pH=8.0) were mixed with 10 µL of the same samples, or standard solutions dissolved in methanol in various thin concentrations (3.125–50 µg/mL) and with 20 µL of BChE or AChE solution in the buffer. The mixtures obtained were placed at 25 °C for 15 min, after which 10 µL of DTNB (0.5 mM) were added. To initiate the reaction, 10 µL of butyrylthiocholine chloride (0.2 mM) were added. The absorbance of the solution was measured at 412 nm using a 96- well microplate reader. Galantamine was used as standard. The formula was used to calculate the percentage of inhibition I (%), and the resulting IC_{50} values (µg/mL) corresponded to the 50% inhibition concentration.

Inhibition % = ((E – S)/ Ac) × 100

where:
Inhibition (%): is the inhibition percentage,
E: is the activity of the enzyme without the sample
S: is the activity of the enzyme with the sample.

2.6. Statistical analysis

Statistical analyses were performed using SPSS 26. Data are presented as mean ± standard deviation (SD) of three independent experiments. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Each oil sample was independently extracted in triplicate (n = 3), and all analytical determinations were performed in three independent experiments to ensure reproducibility. The sample size was selected based on established practices in similar oil extraction and bioactivity studies to ensure statistical reliability

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties

The physicochemical parameters of chia, linseed, and rapeseed oils (Table 2) showed distinct variability in stability and quality indicators. Linseed oil presented the highest refractive index (1.4823), followed by chia (1.4770) and rapeseed oils (1.4674). Since the refractive index is positively correlated with unsaturation, these values confirm linseed oil's higher PUFA content, consistent with Ano et al. (2019). Chia and rapeseed oils exhibited similar refractive indices, in agreement with Nehra and Gahlawat (2022). All oils exhibited comparable densities (~0.92 kg/dm³), consistent with Miller *et al.,* (2022), which reflects the predominance of unsaturated fatty acids that have lower density compared to saturated fats.

TABLE 2. Physicochemical parameters of chia seed, linseed, and rapeseed oils

Physicochemical Parameters	Chia seed oil	Linseed oil	Rapeseed oil
Refractive index (nD, 20 °C)	1.4770 ± 0.0006^{b}	1.4823 ± 0.00016^{a}	1.4674 ± 0.0001^{c}
Density (kg/dm^3)	0.924 ± 0.001^{a}	0.922 ± 0.001^{a}	0.922 ± 0.001^{a}
Acid values (mg KOH/g of oil)	0.56 ± 0.11^{b}	0.78 ± 0.11^{a}	0.74 ± 0.13^{a}
Saponification index (mg KOH/g of oil)	207.00 ± 0.49^{a}	198.96 ± 0.32^{b}	171.00 ± 0.16^{c}
Peroxide value (meq O_2/kg of oil)	1.16 ± 0.28^{c}	2.00 ± 0.50^{b}	3.66 ± 0.57^{a}
Ester index (mg KOH/g of oil)	206.44 ± 0.50^{a}	198.18 ± 0.20^{b}	170.26 ± 0.10^{c}

Values are means (n = 3): Different superscript letters (a–d) in the same line indicate significant differences ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD test. nD = Refractive index at 20 °C; KOH = potassium hydroxide; meq O_2/kg = milliequivalents of oxygen per kilogram of oil.

Acid values were the lowest in chia oil (0.56 mg KOH/g), compared to rapeseed (0.74) and linseed oils (0.78). Lower acid values indicate superior oil quality with fewer free fatty acids and less hydrolytic degradation. Our results are consistent with Rodríguez *et al.,* (2025), who reported acid values of 0.5–1.0

mg KOH/g for chia oil, and Oomah *et al.,* (2009), who noted values of 0.7–1.5 mg KOH/g for linseed oil.

Saponification values were the highest in chia oil (207 mg KOH/g), intermediate in linseed (198.96 mg KOH/g), and the lowest in rapeseed oil (171 mg KOH/g). Higher saponification values indicate shorter-chain fatty acids, as previously described for chia by Timilsena *et al.,* (2017). Rapeseed oil's lower value is consistent with the presence of long-chain fatty acids, in line with Kumar *et al.,* (2022).

Peroxide values, an indicator of oxidative rancidity, were the lowest in chia oil (1.16 meq O_2/kg), moderate in linseed (2.00 meq O_2/kg), and the highest in rapeseed oil (3.66 meq O_2/kg). These findings support chia oil's superior oxidative stability, in agreement with Guiotto *et al.,* (2025). Similarly, ester indices were higher in chia (206.44 mg KOH/g) and linseed oils (198.18 mg KOH/g), compared to rapeseed (170.26 mg KOH/g), confirming a higher proportion of esterified fatty acids in chia and linseed oils, as previously reported by Segura-Campos *et al.,* (2014).

Together, these data suggest that chia oil is the most stable and highest quality, while rapeseed oil exhibits lower stability but contains distinct fatty acids that confer other nutritional advantages. Taken together, these physicochemical variations reflect differences in fatty acid unsaturation and chain length, which are known to influence both oxidative stability and biological functionality (Choe and Min 2006), providing a structural basis for the differences in bioactivities discussed in later sections.

3.1.1. Fatty acid profiles (GC-FID)

The fatty acid compositions of the oils (Table 3, Figure 1) showed marked differences. Chia oil was dominated by α-linolenic acid (C18:3 n-3; 63.30%), followed by linoleic acid (18.20%) and oleic acid (7.29%). Linseed oil also contained high proportions of α-linolenic acid (56.22%), oleic acid (19.32%), and linoleic acid (15.07%). Both oils therefore represent important dietary sources of PUFAs.

TABLE 3. Fatty acid composition (%) of chia seed, linseed and rapeseed oils as determined by GC-FID

			Chia Seed Oil		Linseed Oil		Rapeseed Oil	
Peak	**Name**	**Saturation status**	**Retime**	**Area %**	**Retime**	**Area%**	**Retime**	**Area %**
13	Palmitic acid (C16:0)	SFA	16.266	6.93531	16.267	5.46408	16.218	3.94254
18	Stearic acid (C18:0)	SFA	21.394	4.00891	21.354	3.92084	21.354	1.65691
19	Oleic acid (*cis* C18:1)	MUFA	22.094	7.28613	22.098	19.32278	22.284	64.35396
22	Linoleic acid (*cis* C18.2)	PUFA	23.506	18.19668	23.478	15.06751	23.533	19.05882
25	α-Linolenic acid (C18.3 n3)	PUFA	25.482	63.29973	25.442	56.22479	25.345	9.49457
26	Eicosanoic acid (C20:2)	PUFA	26.118	0.19169	-	-	26.173	1.49320
34	Lignoceric acid (C24)	SFA	30.907	0.08156	-	-	-	-
	Omega-6/Omega-3 ratio*			0.29		0.27		2.01

* ω-6/ω-3 ratio calculated as the percentage of linoleic acid divided by α-linolenic acid.

The high α-linolenic acid content in chia and linseed oils is of particular importance, as ALA contributes to cardioprotective, immunomodulatory, and anti-inflammatory effects (Poudyal *et al.,* 2011). Linoleic acid, abundant in both oils, is known to lower LDL cholesterol and reduce cardiovascular risk (Simopoulos, 2004). The lower oleic acid in chia oil (7.29%) compared to linseed oil (19.32%) indicates a stronger PUFA predominance in chia: whereas linseed offers a more balanced MUFA/PUFA ratio, which can improve oxidative stability and dietary versatility (Li *et al.,* 2025).

Rapeseed oil, in contrast, was dominated by oleic acid (64.35%), with moderate levels of linoleic (19.06%) and α-linolenic acids (9.49%). This MUFA-rich profile enhances oxidative stability and explains its suitability for high-temperature cooking (Davis *et al.,* 2016). Minor fatty acids such as eicosanoic acid (1.49%) were also detected in rapeseed oil, while lignoceric acid, found in chia, was absent.

The calculated omega-6/omega-3 ratios were 0.29, 0.27, and 2.01 for chia seed, linseed, and rapeseed oils, respectively. The low ratios observed in chia seed and linseed indicate a predominance of α-linolenic acid, which is nutritionally favorable. A lower ω-6/ω-3 ratio is generally considered beneficial, as it is associated with reduced inflammatory responses and a lower risk of autoimmune diseases, asthma, and allergies. It

also contributes to improved metabolic function and overall health by supporting cardiovascular protection, reducing insulin resistance, inhibiting tumor growth, and alleviating neuroinflammatory and depressive symptoms (Li *et al.*, 2024). The ratios observed in chia seed and linseed (< 1.0) suggest a favorable fatty acid balance compared to many commonly consumed vegetable oils.

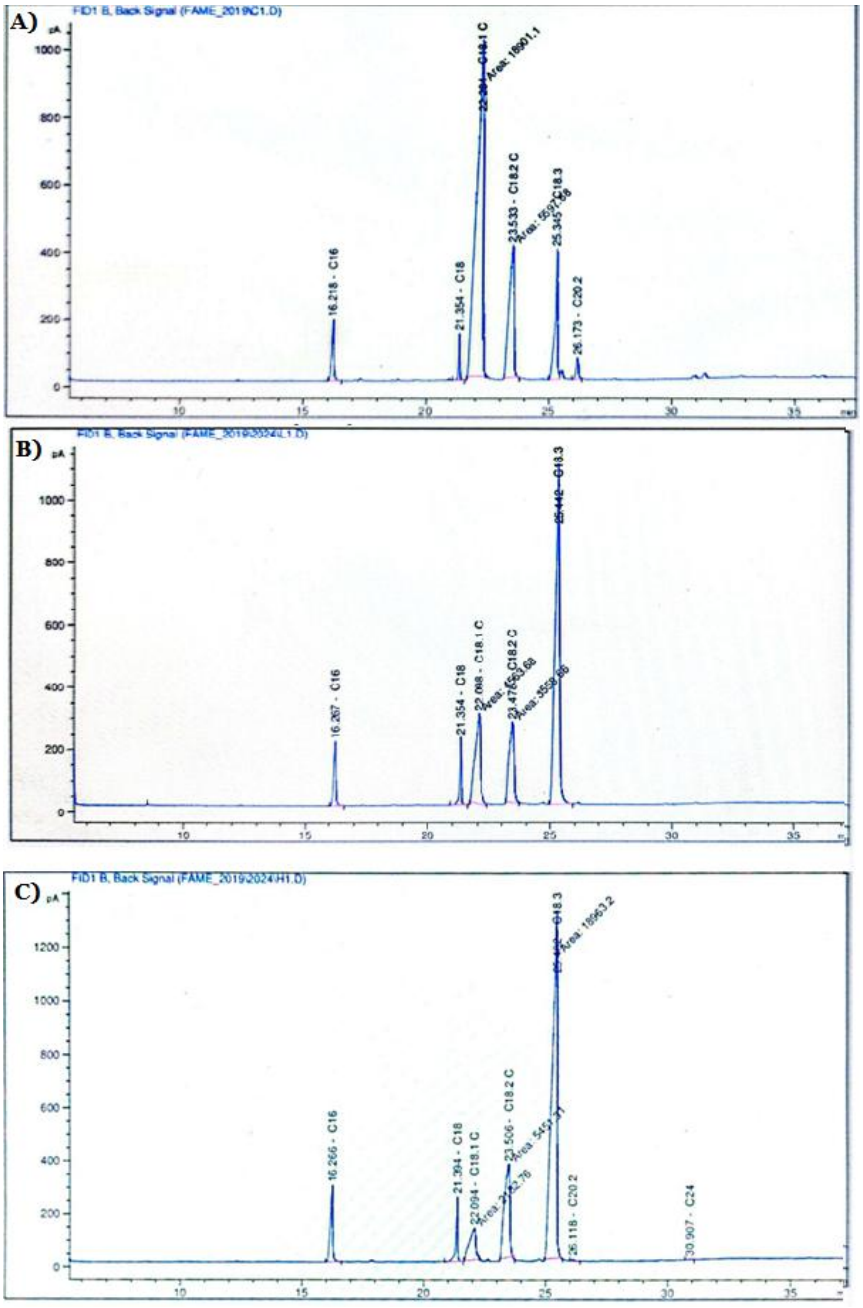

FIGURE 1. Fatty acid profiles of rapeseed (A), linseed (B) and chia seed oils determined by GC-FID

Overall, chia and linseed oils stand out as PUFA-rich sources with strong nutritional and therapeutic potential, while rapeseed oil provides stability and functional culinary benefits due to its high oleic acid content.

3.1.2. Bioactivities: enzyme inhibition and anti-inflammatory effects

The inhibitory activities of the oils (Table 4, Figure 2) revealed distinctive patterns.

TABLE 4. Inhibitory activities (IC_{50}, µg/mL) of chia seed, linseed, and rapeseed oils against acetylcholinesterase, butyrylcholinesterase, and protein denaturation (anti-inflammatory)

Sample	Acetylcholinesterase Inhibition (IC_{50}, µg/mL)	Butyrylcholinesterase Inhibition (IC_{50}, µg/mL)	Anti-inflammatory Activity (IC_{50}, mg/mL)
Chia seed oil	16.44 ± 0.38^{b}	49.83 ± 2.01^{b}	2.46 ± 0.05^{b}
Linseed oil	18.82 ± 1.00^{c}	52.60 ± 3.44^{b}	4.00 ± 0.08^{d}
Rapeseed oil	34.74 ± 0.25^{d}	75.21 ± 3.74^{c}	3.61 ± 0.06^{c}
Galantamine	6.27 ± 1.15^{a}	34.75 ± 1.99^{a}	–
Diclofenac	–	–	1.88 ± 0.01^{a}

Values are expressed as mean ± SD (n = 3). Different superscript letters (a–d) in the same column indicate significant differences ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD test : IC_{50} = half maximal inhibitory concentration; SD = standard deviation.

For acetylcholinesterase (AChE), chia oil exhibited the strongest inhibition (IC_{50} = 16.44 µg/mL), followed by linseed (18.82 µg/mL) and rapeseed (34.74 µg/mL). Although galantamine (6.27 µg/mL) was more potent, the oils showed significant inhibitory activity, most likely due to their PUFA and phenolic contents, which have been linked to neuroprotective effects (Moliner *et al.,* 2018).

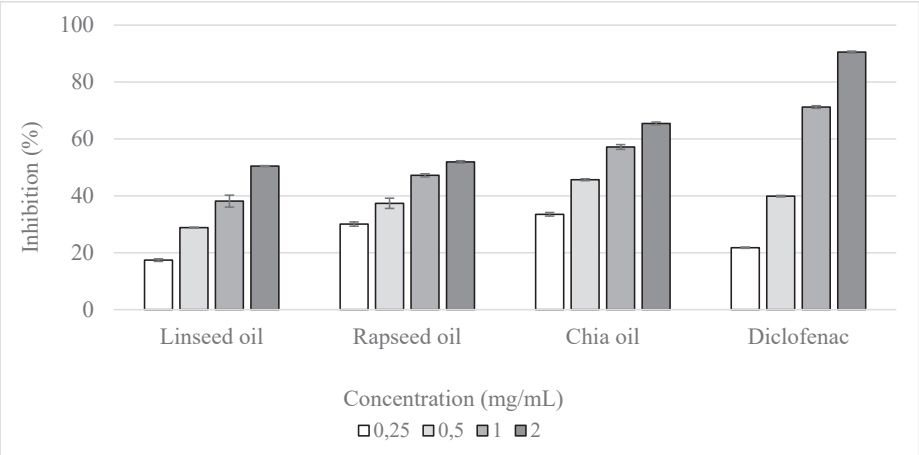

FIGURE 2. Dose-dependent anti-inflammatory activity of chia seed, linseed, and rapeseed oils compared to diclofenac

Values are expressed as mean ± SD (n = 3). Different letters indicate significant differences between samples ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD test**:** SD = standard deviation.

For butyrylcholinesterase (BChE), chia oil (49.83 µg/mL) and linseed oil (52.60 µg/mL) showed greater activity compared to rapeseed oil (75.21 µg/mL).

The higher inhibitory potential of chia and linseed oils reinforces their promise as natural anti-Alzheimer's agents. In terms of anti-inflammatory activity, measured by the inhibition of protein denaturation, linseed (IC_{50}= 4.00 µg/mL) and rapeseed oils (3.61 µg/mL) were less active than chia (2.46 µg/mL), although diclofenac (1.88 µg/mL) was superior. Linseed oil's strong effect is consistent with its omega-3 and polyphenol contents (Grancieri *et al.,* 2019), while rapeseed oil's activity may derive from its MUFA fraction and vitamin E (Bagherniya *et al.,* 2021). Chia oil's moderate inhibition can be attributed to its PUFA and antioxidant composition.

The relatively stronger inhibition observed for chia and linseed oils may be directly attributed to their elevated αlinolenic acid levels, which have been shown to modulate neuroinflammatory pathways and influence neural cell membrane composition and function (Fourrier et al., 2017). Dietary ω3 polyunsaturated fatty acids, of

which ALA is a precursor, can regulate microglial activation and inflammatory signaling in the central nervous system, contributing to neuroprotective effects (Desale and Chinnathambi, 2020). In addition, αlinolenic acid has been reported to inhibit acetylcholinesterase activity in vitro, further supporting the hypothesis that ω3–rich lipid matrices contribute to enhanced cholinesterase inhibition (Akay et al., 2023).

These results indicate that chia and linseed oils are more promising in neuroprotection through cholinesterase inhibition, while linseed and rapeseed oils are more effective as anti-inflammatory agents.

3.1.3. Integrated interpretation

Overall, the three seed oils demonstrated complementary physicochemical and bioactive profiles. Chia and linseed oils, with their exceptionally high α-linolenic and linoleic acid content, are potent sources of PUFAs with strong neuroprotective potential, but are more prone to oxidation. Rapeseed oil, dominated by oleic acid, is more oxidatively stable and provides superior anti-inflammatory activity, making it highly suitable for both dietary and industrial applications.

The integration of these findings suggests potential strategies for nutraceutical and functional food applications, either through targeted use of individual oils or by blending them to combine stability with enhanced bioactivity.

One limitation of this study is the absence of phenolic compound quantification, despite its recognized importance in determining the biological and antioxidant properties of vegetable oils. Although the phenolic content of these oils is generally lower than that of olive oil, its contribution to oxidative stability and potential health benefits should not be overlooked. In addition, the inclusion of further oxidative markers, such as conjugated dienes and TBARS, would provide deeper insight into the formation of secondary oxidation products. Future studies are therefore warranted to investigate the oxidative stability of these oils in greater detail, as well as to analyze and quantify their phenolic fractions and evaluate their contribution to overall oxidative stability.

4. CONCLUSIONS

This study highlights the distinct physicochemical and biological characteristics of cold-pressed chia, linseed, and rapeseed oils. Chia seed oil exhibited superior oxidative stability and high ester content, indicating excellent quality and potential health benefits. Linseed oil showed a favorable balance of polyunsaturated fatty acids and biological activity, making it a valuable nutritional source. Rapeseed oil, rich in monounsaturated fatty acids, demonstrated good oxidative stability and suitability for culinary applications. The biological assays suggest that chia and linseed oils possess significant anti-inflammatory and neuroprotective potential, supporting their use as natural complementary agents in managing inflammation and neurodegenerative disorders.

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AUTHORS' CONTRIBUTIONS

S.L. and S.B. conceptualized and designed the study. S.L., S.A., and C.B. carried out the experimental work and data acquisition. R.E. and F.B. contributed to the methodology and analytical validation. F.A.A. and M.A.W. provided critical insights into the biological activity evaluations and

revised the manuscript for important intellectual content. L.S. and A.A.A contributed to data interpretation and language editing. A.A.A and S.B. secured funding, and finalized the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

ETHICAL APPROVAL

Not applicable for this study as it did not involve human participants or animals.

ABBREVIATIONS

The following abbreviations are used throughout the manuscript: **AChE** (acetylcholinesterase), **AFNOR** (Association Française de Normalisation), **AI** (acidity index), **ALA** (alpha-linolenic acid), **ANOVA** (analysis of variance), **BChE** (butyrylcholinesterase), **BSA** (bovine serum albumin), **DTNB** (5,5′-dithiobis-(2-nitrobenzoic acid)), **EI** (ester index), **FID** (flame ionization detector), **GC** (gas chromatography), **ALA** (alpha-linolenic acid), **IC_{50}** (half maximal inhibitory concentration), **KOH** (potassium hydroxide), **MUFA** (monounsaturated fatty acids), n-3 (omega-3), n-6 (omega-6), **PI** (peroxide index), **PUFA** (polyunsaturated fatty acids), **RI** (refractive index), and **SV** (saponification value), **SFA**: Saturated fatty acids (no double bonds)

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