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Research article

Comparative fatty acid profiling of seed lines of hemp (*Cannabis sativa* L.) and inhibition of tyrosinase, α-glucosidase and acetylcholinesterase

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Abstract

Importance of the work: Hemp (*Cannabis sativa* L.) has gained renewed interest for its potential in food and medicinal products, though its seeds remain underutilized. This study is the first to evaluate the fatty acid composition of hemp seeds using FAME analysis. It also examines the tyrosinase, α-glucosidase and acetylcholinesterase inhibitory activity of extracts from different hemp lines.

Objectives: To analyze the fatty acid composition and assess the levels of tyrosinase, α-glucosidase and acetylcholinesterase inhibitory activity of various hemp seed lines for potential industrial applications.

Materials and Methods: The fatty acid composition was determined based on FAME analysis with gas chromatography-mass spectrometry that provided fast and accurate assessment. The levels of tyrosinase, acetylcholinesterase and α-glucosidase inhibitory activity were evaluated to determine the percentage of inhibition.

Results: Over 50% of the fatty acids in the hemp seed extracts were saturated fatty acids, with palmitic acid being predominant. The distribution of polyunsaturated fatty acids, including omega-3 and omega-6, and of monounsaturated fatty acids varied across lines, though there were no significant differences between lines E and G. Tyrosinase inhibitory activity varied significantly, with hemp line A producing the highest mean inhibition (29.2 ± 4.0%), while the others were in the range 11.1–29.1%. There were no significant differences in acetylcholinesterase inhibition across lines, ranging from 26.7 ± 1.0% to 28.3 ± 0.8%. Notably, all hemp seed extracts had high α-glucosidase inhibition, ranging from 97.0 ± 1.3% to 101.9 ± 1.0%.

Main finding: This was the first report that the fatty acid composition across different hemp seed lines was not significantly different, which aligned with the consistent levels of acetylcholinesterase inhibition observed. However, there were notable differences in the levels of tyrosinase inhibition among the lines. Notably, all the hemp seed extracts had highly potent levels of α-glucosidase inhibition.

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Introduction

Hemp (*Cannabis sativa* L.) is a versatile plant with a wide range of uses and has been cultivated for thousands of years. Despite its benefits, its widespread use is now restricted due to the presence of Δ^9-tetrahydrocannabinol (THC) in its flowers and leaves. However, many countries have begun lifting their bans on hemp usage, leading to a resurgence in interest due to its potential as a source of food and medicinal products (Xu et al., 2022). While hemp flowers and leaves are known for their medicinal properties, the hemp seeds that are primarily composed of oil, are still rarely used (Leizer et al., 2000). This oil is rich in essential fatty acids, including omega-3 and omega-6, crucial for maintaining human health (Anwar et al., 2006; Harris et al., 2008). Despite its nutritional benefits, hemp seed oil remains underutilized in the industrial sector, particularly in cosmetics and food production. Exploring new applications for hemp seed oil could lead to major advancements in these industries, promoting the sustainable use of natural resources.

Fatty acids are essential components in both the food and cosmetic industries. In the food supplement industry, they are valued for their nutritional benefits and role in maintaining a healthy diet because essential fatty acids, which are not synthesized in the human body, must be obtained through the diet, with hemp seed oil being an excellent source (Razmaitė et al., 2021). A recent study suggested that omega-3 and omega-6 fatty acids exhibited acetylcholinesterase (AChE) inhibitory activity (Akay et al., 2023), potentially contributing to the prevention of Alzheimer's disease (AD). Acetylcholinesterase inhibitors (AChEIs) are a prominent strategy in developing disease treatments for AD (Terry et al., 2003). Existing evidence points to AChE inhibitory activity of hemp-derived compounds, such as lignanamides (Yan et al., 2015) and cannabinoids (Furqan et al., 2020; Puopolo et al., 2022), indicating that hemp seed oil could also have similar properties. Furthermore, studies have reported α-glucosidase inhibitory activity in cannabidiol (CBD) and THC from the leaves of *C. sativa* (Ma et al., 2021; Suttithumsatid et al., 2022), as well as oligopeptides from hemp seeds (Ren et al., 2016) that help to regulate postprandial blood glucose levels (Yen et al., 2021).

In the cosmetic industry, fatty acids are also prized for their moisturizing and anti-inflammatory properties, which help maintain skin health and appearance (Calder, 2010; Kelm et al., 2017). The high content of essential fatty acids in hemp seed oil could offer natural solutions for various skin conditions in skincare products. In addition, essential fatty acids have produced significant inhibitory activity against tyrosinase (Tel-Çayan et al., 2013; Balcos et al., 2014; Kose, 2023). Substances inhibiting tyrosinase activity are highly sought in the cosmetic industry for their potential to treat hyperpigmentation and skin tone (Hassan et al., 2023). The search for natural tyrosinase inhibitors has led researchers to investigate various plant extracts, including hemp seed oil, which may offer effective and safe alternatives to synthetic compounds. For example, there is promising evidence for tyrosinase inhibition by hemp leaf and hemp seed extracts (Manosroi et al., 2019).

The above-mentioned reports provide some information on the enzyme inhibitory activity of the flowers and leaves of hemp; however, there been little reported on the activity of the seeds. Furthermore, no research exists comparing fatty acid composition and enzyme inhibition between hemp seed lines. Thus, the current report investigated the fatty acid profiles of hemp seed extracts from different lines and their tyrosinase, α-glucosidase and acetylcholinesterase inhibitory activities to identify the most promising varieties for industrial use. These findings could help raise the value of hemp seeds, providing new economic opportunities for farmers and contributing to the sustainable development of the agricultural sector.

Materials and Methods

Materials

All solvents used in this study were purified following standard procedures. Tyrosinase (EC 1.14.18.1) from mushroom, α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, acetylcholinesterase (EC 3.1.1.7) from *Electrophorus electricus* and 3,4-dihydroxy-L-phenylalanine (L-DOPA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodine (ATCI) and tacrine were purchased from Sigma-Aldrich (USA). Kojic acid, nitrophenyl-α-D-gluco-pyranoside (PNP-G) and acarbose hydrate were sourced from TCI (Japan). Sodium carbonate was purchased from Kemaus (Australia). Dimethyl sulfoxide (DMSO) was purchased from Carlo Erba (France).

Hemp seed collection

Hemp seeds were collected from exceptional hemp plants from local planting sites in northern Thailand and their seed weight,

length and width were recorded. Eight hemp lines that produced high seed yield in different areas were selected to investigate potential applications. The average weight ± SD of the seeds was 3.02 ± 0.31 g per 100 seeds, with values for mean seed width and seed length of 3.52 ± 0.21 mm and 4.94 ± 0.19 mm, respectively (Unpublished data).

Preparation of hemp seed extracts

Approximately 30 g of seeds from each line were sun-dried and then ground into a fine powder using a grinder. The resulting powder was transferred to a flask for extraction based on maceration. Each ground sample was soaked in 300 mL of ethanol for 7 d. After the soaking period, the extracts were passed through a vacuum filtration system. Next, the ethanol solvent was evaporated to dryness using a rotary evaporator and high vacuum, yielding the crude extract. The crude extract was weighed to determine the yield for each line. Finally, all extracts were stored at 4°C in a refrigerator.

Preparation of fatty acid methyl ester

The fatty acid composition was determined based on a gas chromatographic method and fatty acid methyl ester (FAME) analysis. The methods and conditions of preparation were set up according to Buthelezi et al. (2012) and Srisuksai et al. (2024), with slight modifications. Initially, 20 mg of hemp seed oil was added into separate 15 mL centrifugal tubes; then, 3 mL of BF_3-MeOH solution was added. The mixture was heated in a water bath at 70°C for 30 min. After cooling, 1 mL of *n*-hexane and 600 µL of distilled water were added and mixed thoroughly. The organic phase was separated using centrifugation at 4,500 rpm for 5 min, followed by filtering using a Millipore 0.45 mm. A 1 µL aliquot was injected for gas chromatography.

Gas chromatography-mass spectrometry analysis of fatty acid methyl ester

The FAME analysis was performed using a gas chromatography-mass spectrometer (GCMS-QP2020; Shimadzu, Kyoto, Japan) with a FAME column (ZB-FAME; Zebron; Newport Beach, CA, USA) with a length of 30 m, an internal diameter of 0.25 mm and a stationary phase film thickness of 0.20 µm. The oven temperature program started at 90°C, increased to 160°C at a rate of 10°C/min, was held for 5 min, then increased to 170°C at the rate of 2°C/min, held for 7 min and increased to 200°C at the rate of 8°C/min and then held for 5 min for a total analysis time of 39.75 min. A split/spitless injector was used with an injector temperature of 240°C and a split ratio of 50:1. Helium was used as a carrier gas, with a flow rate of 1.0 mL/min. The injected volume was 1 µL, with a solvent delay of 3.5 min and a mass spectrometer ionization voltage of 70 eV. Data were collected in SCAN mode. The FAMEs were identified by comparing their mass spectral and fragmentation patterns with those in the NIST library. When searching NIST, the match factors for both sources produced similar results, with most compounds having scores above 900 (indicating an excellent match). The FAME contents were expressed as weight percentages. Samples were prepared separately in triplicate and average values were presented as results.

Tyrosinase inhibitory activity

The tyrosinase inhibitory activity of the hemp seeds extract was evaluated using the dopachrome method with L-DOPA as a substrate. (Mutschlechner et al., 2018) The hemp seed crude extracts were dissolved in DMSO and diluted with distilled ionized (DI) water to yield the corresponding test concentrations (with the final concentration consisting of less than 1% DMSO volume per volume; v/v). The experiment was conducted using 96-microtiter well plates, which included a blank, a test and a test control.

In the test group, each well received 100 µL of 0.02 M phosphate buffered saline (PBS) at pH 6.8, 40 µL of crude sample and 20 µL of 50 U/mL tyrosinase solution. The plates were incubated for 10 min at 23°C. After incubation, 40 µL of L-DOPA was added and incubated for an additional 5 min. In the test control group, 20 µL of PBS at pH 6.8 was used instead of 20 µL of 50 U/mL tyrosinase solution. In the blank group, 40 µL of DI water was used to replace the 40 µL of the sample. Kojic acid was used as a positive control. The absorbance was measured at a wavelength of 475 nm using a microplate absorbance reader (MPR; Spark™ 10M) and the percentage inhibition was calculated using Equation 1:

$$\text{Inhibition (\%)} = 1 - (\text{Absorbance of sample} / \text{Absorbance of control}) \times 100 \quad (1)$$

where the absorbance of the sample is the absorbance in a test group minus a test control and the absorbance of the control is minus the absorbance in a blank. All experiments were performed in triplicate and data were expressed as mean ± SD.

Acetylcholinesterase inhibitory activity

The acetylcholinesterase inhibitory activity of each hemp seed extract was evaluated based on Ellman et al. (1961), following the protocol of Tharamak et al. (2023). The hemp seed extracts were dissolved in DMSO and then diluted with DI water to achieve a test concentration (with a final DMSO concentration of less than 1% v/v). The assay was conducted in 96-well microtiter plates, containing a blank, a test and a test control.

For the test group, 90 µL of PBS (pH 8.0), 10 µL of extract, 20 µL of 0.4 U/mL AChE solution and 40 µL of 0.6 mmol/L DTNB were added to each well and incubated for 10 min at 37°C. Subsequently, 40 µL of 0.6 mmol/L ATCI as substrate was added and incubated for an additional 20 min. In the test control group, 20 µL of buffer (pH 8.0) replaced the 20 µL of 0.4 U/mL AChE solution. In the blank group, 10 µL of DI water was substituted for the 10 µL of the extract. Tacrine served as the positive control. The absorbance was measured at 412 nm using a microplate reader (MPR; Spark™ 10M), and the percentage inhibition was calculated using Equation 1.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the hemp seed extract was evaluated following the protocol of Pluempanupat et al. (2007) For the test group, 50 µL of 0.1 M PBS (pH 6.8), 10 µL of crude extract (final concentration of 0.50 mg/mL with less than 1% v/v DMSO) and 20 µL of 0.2 U/mL α-glucosidase from *Saccharomyces cerevisiae* were combined. After incubation at 37°C for 5 min, 20 µL of 2.5 mM PNP-G was added as a substrate. The reaction was further incubated at 37°C for 15 min and then stopped by adding 50 µL of 0.4 M Na_2CO_3. In the test control group, 20 µL of buffer (pH 6.8) replaced the 20 µL of enzyme solution. In the blank group, 10 µL of DI water was substituted for the 10 µL of the crude extract. Acarbose was used as a positive control. Absorbance was measured at 400 nm using a microplate reader (MPR; Spark™ 10M), and the percentage inhibition was calculated using Equation 1.

Statistical analysis

Statistical analysis was conducted on data obtained from triplicate experiments, with results expressed as mean ± SD. To identify significant differences between means, the data were analyzed using one-way analysis of variance followed by Tukey's post hoc test. Significance was tested at the $p < 0.05$ level. All analyses were performed using R Statistical Software (v4.1.2; R Core Team, 2021; https://www.r-project.org/).

Results and Discussion

Extraction of different hemp seeds

Extraction is a common method for obtaining essential compounds from plants. The current study extracted substances from eight lines of hemp seeds, which produced high seed yields, by soaking them in ethanol. Based on the results, the percentage yields of the hemp seed extracts were in the range 4.82–9.12% weight per weight, as shown in Table 1. Each extract was characterized as a viscous fluid with a dark brown color.

Table 1 Locations and crude extract yields of eight hemp seed lines (A-H) in Thailand

Line	Location	District	Province	Yield (% w/w)
A	Samoeng Tai	Samoeng	Chiang Mai	6.12
B	Mae Ramat	Mae Ramat	Tak	5.10
C	Mae Sot	Mae Sot	Tak	4.82
D	Khun Yuam	Khun Yuam	Mae Hong Son	5.20
E	Tha Sai Luat	Mae Sot	Tak	9.12
F	Sop Moei	Sop Moei	Mae Hong Son	6.72
G	Mae Ramat	Mae Ramat	Tak	5.56
H	Mueang Pon	Khun Yuam	Mae Hong Son	8.90
Mean				6.44
Maximum				9.12
Minimum				4.82
SD				1.70

w/w = weight per weight

Composition of fatty acids in hemp seed extracts

Fatty acids play a crucial role in various industries, including food, cosmetics and pharmaceuticals (Calder, 2010; Tel-Çayan et al., 2013; Balcos et al., 2014; Kelm and Wickett, 2017; Razmaitė et al., 2021; Kose, 2023). Determining the fatty acid composition in natural extracts, such as those from hemp seeds, enhances their value and provides essential information for potential industrial applications. This knowledge serves as a valuable resource for entrepreneurs, guiding the development of new products and optimizing the use of these natural extracts. Identifying the specific fatty acid profiles can inform targeted applications, ensuring that the extracts are used in the most effective and beneficial ways.

The fatty acid composition of extracts from the eight different hemp seed lines was analyzed using gas chromatography-mass spectrometry and the FAME method. Evaluation involved the three main types of fatty acids: saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), with the resultant percentages displayed in Fig 1A. Based on these results, SFAs accounted for over 50% of the total fatty acids, and the amount was significantly different to the amounts of MUFAs and PUFAs. The MUFAs and PUFAs were the second most abundant, with ranges of 21.5–31.2% and 7.0–17.1%, respectively. Notably, the distribution of MUFAs and PUFAs varied among the different lines. Six hemp lines (A, B, C, D, F and H) had significantly higher MUFA percentages than the PUFAs. In contrast, there was no significant difference between the PUFA and MUFA levels for lines E and G. Furthermore, among the SFAs, line B had the highest content; however, this was not significantly different from the amounts from lines E, H, F, C and G. In addition, lines A and D had significantly lower SFA levels than line B. There were no significant differences among the PUFAs and MUFAs across all lines, as illustrated in Fig. 1B.

Plants of the same species often produce varying quantities and qualities of compounds when cultivated in different environments, necessitating the ongoing search for optimal growing conditions (Li et al., 2020). However, in the current study, there were no significant differences in the fatty acid content among the different hemp lines cultivated in Thailand, as presented in Table 2. This uniformity in fatty acid composition could be advantageous for industrial applications, where consistency in product quality is often considered essential. The detailed fatty acid analysis revealed that the most abundant fatty acid across all hemp seed extracts was palmitic acid (C16:0), contributing approximately 34.2–41.8% of the total fatty acid content. Stearic acid (C18:0) was the second most prominent, constituting 16.0–20.2% of the total fatty acid content across all lines. The high content of SFAs (predominantly palmitic and stearic acids) in the hemp seed extracts suggested high stability with potential applications in various industries, particularly in the formulation of stable cosmetic and food products.

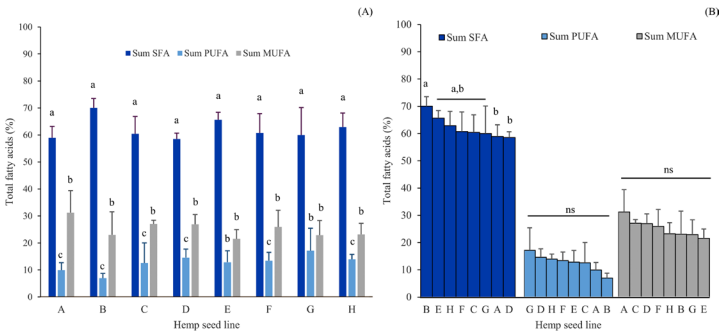

Fig. 1 Percentages of total fatty acids in hemp seed extracts from eight hemp lines (A–H), with error bars representing ± SD from triplicate experiments: (A) summary (Sum) of total amounts of saturated (SFA), polyunsaturated (PUFA), and monounsaturated (MUFA) fatty acids in each hemp seed extract, with lowercase letters above bars indicating significant ($p < 0.05$) differences within same hemp seed line; (B) sorted total fatty acid content across hemp seed extracts, with different lowercase letters above bars indicating significant differences and ns indicating non-significant ($p \geq 0.05$) differences

Table 2 Percentages of each fatty acid in hemp seed extracts from eight hemp lines (A–H)

Fatty acid	Common name	Type	Fatty acid composition (%)							
			A	B	C	D	E	F	G	H
C16:0	Palmitic acid	SFA	34.9 ± 2.2^{a}	41.8 ± 1.9^{a}	34.2 ± 2.1^{a}	35.7 ± 0.6^{a}	38.5 ± 1.3^{a}	35.3 ± 4.1^{a}	35.7 ± 5.7^{a}	35.4 ± 0.9^{a}
C18:0	Stearic acid	SFA	18.2 ± 1.6^{a}	17.7 ± 0.7^{a}	16.0 ± 0.8^{a}	16.0 ± 0.3^{a}	20.2 ± 0.7^{a}	16.6 ± 2.0^{a}	16.6 ± 3.0^{a}	18.5 ± 0.7^{a}
C18:1 n-9	Oleic acid	MUFA	18.1 ± 3.6^{a}	17.1 ± 6.1^{a}	15.2 ± 0.2^{a}	15.0 ± 3.5^{a}	11.6 ± 2.9^{a}	13.4 ± 1.3^{a}	11.2 ± 2.9^{a}	14.2 ± 1.2^{a}
C18:1 n-12	Petroselinic acid	MUFA	13.1 ± 4.6^{a}	5.9 ± 2.4^{a}	11.9 ± 1.2^{a}	11.9 ± 0.1^{a}	9.9 ± 0.6^{a}	12.5 ± 4.9^{a}	11.7 ± 2.6^{a}	9.0 ± 2.9^{a}
C18:2 n-6	Linoleic acid	PUFA	6.3 ± 0.9^{a}	4.9 ± 1.2^{a}	8.0 ± 4.4^{a}	10.9 ± 2.2^{a}	9.8 ± 3.1^{a}	8.9 ± 1.0^{a}	12.9 ± 6.5^{a}	9.5 ± 0.7^{a}
C18:3 n-3	α-Linolenic acid	PUFA	1.5 ± 0.2^{a}	1.1 ± 0.5^{a}	1.6 ± 1.8^{a}	1.8 ± 0.4^{a}	1.1 ± 0.5^{a}	1.8 ± 0.9^{a}	1.7 ± 0.6^{a}	2.4 ± 1.0^{a}
C20:3 n-3	Eicosatrienoic acid	PUFA	2.0 ± 1.7^{a}	1.0 ± 0.1^{a}	3.0 ± 1.3^{a}	1.9 ± 0.6^{a}	1.9 ± 0.7^{a}	2.6 ± 1.2^{a}	2.5 ± 1.2^{a}	2.1 ± 0.2^{a}
C22:0	Behenic acid	SFA	1.6 ± 0.1^{a}	2.0 ± 0.0^{a}	3.6 ± 3.1^{a}	1.7 ± 0.1^{a}	2.0 ± 0.1^{a}	1.5 ± 0.2^{a}	1.6 ± 0.3^{a}	2.2 ± 0.5^{a}
C24:0	Lignoceric acid	SFA	4.2 ± 0.3^{a}	8.5 ± 0.8^{b}	6.5 ± 0.4^{ab}	5.1 ± 1.1^{ab}	4.9 ± 0.8^{ab}	7.4 ± 0.9^{ab}	6.1 ± 1.1^{ab}	6.8 ± 3.0^{ab}

SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids.
Values (mean ± SD; $n = 3$) in same row with different lowercase superscripts are significantly ($p < 0.05$) different.

The similar levels of PUFAs and MUFAs across the different hemp lines suggested that the environment may have had only a limited impact on fatty acid biosynthesis. PUFAs, including omega-3 and omega-6 fatty acids, are essential for human health and are linked to anti-inflammatory properties and cardiovascular health improvements (Calder, 2010). In the current study, hemp seed extract from line C had the highest concentration of eicosatrienoic acid (an omega-3 fatty acid) at 3.0%. Line H had the highest level of α-linolenic acid (an omega-6 fatty acid) at 2.4%. Other lines contained omega-3 and omega-6 levels in the ranges 1.0–2.6% and 1.1–1.8%, respectively, with no significant differences between lines C and H. These results indicated that despite potential environmental influences, the fatty acid profile in these hemp lines had remained stable, which is advantageous for the consistent production of hemp seed oil in industrial applications.

Tyrosinase inhibitory activity of hemp seed extracts

The ability to inhibit the tyrosinase enzyme is a crucial biological activity in cosmetic products, as it can help manage hyperpigmentation and even skin tone (Hassan et al., 2023). The current evaluated the tyrosinase inhibitory activity of crude extracts from the hemp seeds of different lines using the dopachrome method. The percentage of inhibition was assessed at a concentration of 0.75 mg/mL (as illustrated in Fig. 2) due to solubility issues of the crude extract in aqueous solutions at concentrations above 1 mg/mL.

Based on the results, the hemp seed extracts had tyrosinase inhibition in the range 11.1–29.2%, with line A having the highest inhibition (29.2 ± 4.0%). The statistical analysis revealed no significant differences in inhibitory activity between line A and the second-to-fourth most effective lines (B–E) that had inhibition levels in the range 24.3–29.1%. In contrast, lines F–H) had significantly lower inhibitory activity than line A.

Notably, the tyrosinase inhibitory activity did not correlate with the fatty acid profiles of the hemp seed extracts, suggesting that the active compounds responsible for tyrosinase inhibition were likely distinct from the primary fatty acid components and may have varied in concentration across the different lines. These findings highlighted the presence of important tyrosinase inhibitory substances in hemp seeds that varied in amount between lines. Further research is needed to identify and quantify these active compounds and to explore their potential applications.

Acetylcholinesterase inhibitory activity of hemp seed extracts

AChE is a crucial enzyme that hydrolyzes acetylcholine into choline and acetic acid within the synaptic cleft. AChEIs have become a primary focus in the development of therapeutic agents for Alzheimer's disease due to their ability to increase the acetylcholine levels in the brain (Terry and Buccafusco, 2003). Akay et al. (2023) reported that certain fatty acids (such as omega-3 and omega-6) and Yan et al. (2015) reported that lignanamides from hemp seeds had inhibitory effects on AChE. Consequently, in the current study, we investigated the AChE inhibitory activity of crude extracts from hemp seeds of various lines at a concentration of 0.75 mg/mL using the Ellman method, as illustrated in Fig. 3.

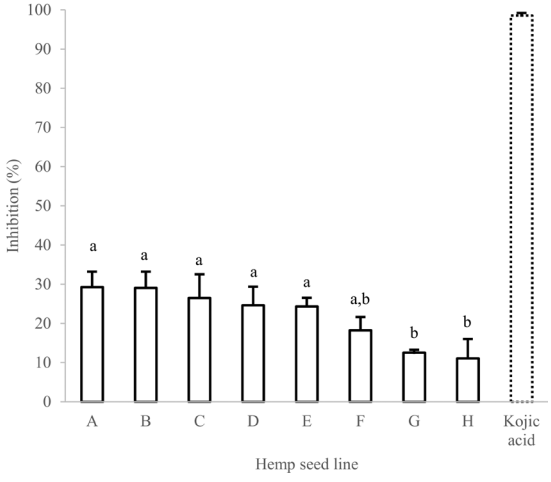

Fig. 2 Percentages of tyrosinase inhibition at 0.75 mg/mL of hemp seed extracts from eight hemp lines (A–H), with error bars indicating ± SD from triplicate experiments; different lowercase letters above bars indicate significant ($p < 0.05$) differences; kojic acid at 1,000 µM used as a positive reference

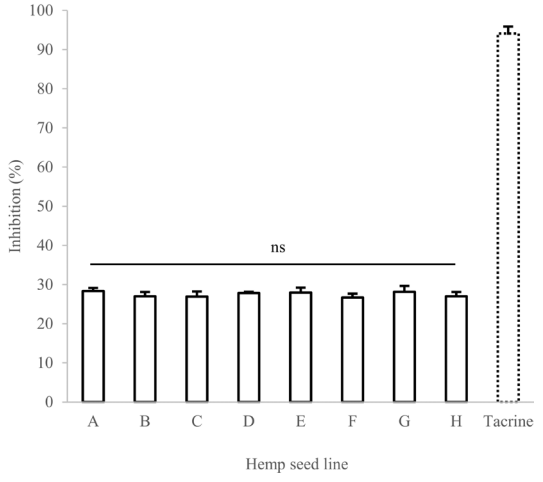

Fig. 3 Percentages of acetylcholinesterase inhibition at 0.75 mg/mL of hemp seed extracts from eight hemp lines (A–H), with error bars indicating ± SD from triplicate experiments; ns indicates non-significant ($p \geq 0.05$) differences; tacrine at 4 µM used as a positive reference

The results showed that the percentage inhibition of acetylcholinesterase by the hemp seed extracts was in the range 26.7 ± 1.0% to 28.3 ± 0.8%, with no significant differences observed among the lines, suggesting that the active compounds responsible for the inhibitory activity could be fatty acids, such as omega-3 and omega-6 as has been reported in the literature (Akay et al., 2023), that were consistently present in similar quantities across the different hemp seed extracts.

α-Glucosidase inhibitory activity of hemp seed extracts

α-Glucosidase is an enzyme crucial for breaking down starches into sugars and inhibiting this enzyme can potentially slow sugar absorption into the bloodstream, offering benefits for patients with type 2 diabetes (Yen et al., 2021). The current study evaluated the α-glucosidase inhibitory activity of crude extracts from various hemp seed lines at a concentration of 0.50 mg/mL, as illustrated in Fig. 4.

All hemp seed extracts had significant levels of α-glucosidase inhibitory activity, with the inhibition percentages ranging from 97.0 ± 1.3% to 101.9 ± 1.0%. Suttithumsatid et al. (2022) reported that THC and CBD, commonly found in hemp, produced α-glucosidase inhibition. However, hemp seeds contained minimal amounts of THC and CBD but were rich in fatty acids (Xu et al., 2022). It was likely that the observed potent α-glucosidase inhibitory activity in the hemp seed extracts in the current study was primarily due to their high fatty acid content. This was consistent with a report indicating that palmitic and oleic acids from *Blechum pyramidatum* were effective α-glucosidase inhibitors (Cherigo and Martínez-Luis, 2018).

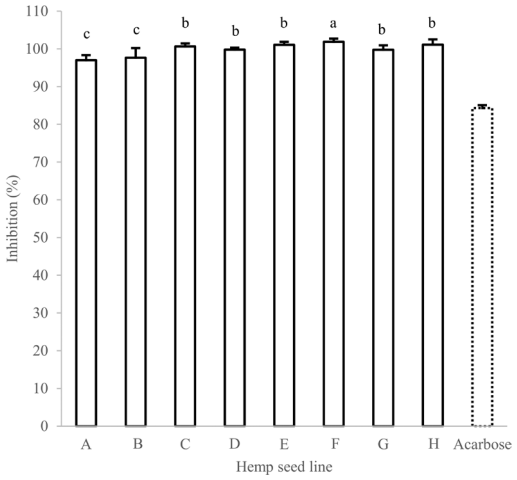

Fig. 4 Percentages of α-glucosidase inhibition at 0.50 mg/mL of hemp seed extracts from eight hemp lines (A–H), with error bars indicating ± SD from triplicate experiments; different lowercase letters above bars indicate significant ($p < 0.05$) differences; acarbose at 1.6 mM used as a positive reference

The current findings supported this conclusion based on the high concentration of palmitic acid in the hemp seed extract,. Further research will be conducted to identify additional active compounds in hemp seeds, aiming to enhance their potential applications in the food and pharmaceutical industries.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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