Future Journal of Pharmaceutical Sciences

RESEARCH

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Exploring of biological activity and diverse metabolites in hemp (*Cannabis sativa*) seed oil by GC/MS, GC–FID, and LC–HRMS chromatographies

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Abstract

Background This study investigated the antidiabetic and antioxidant properties of hemp seed oil using various bioanalytical methods. Furthermore, this study determined the suppressive properties of hemp seed oil on α-amylase, acetylcholinesterase and carbonic anhydrase II that purified by the sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatoghraphy, all of which are related to different metabolic diseases. Moreover, the phenolic concentration in the essential oil was quantified through LC–HRMS chromatography. Thirteen distinct phenolic compounds were detected in hemp seed oil. Additionally, both the chemical components and quantity of essential oils within hemp seed oil were assessed through GC–FID and GC/MS analyses.

Results The predominant essential oils in hemp seed oil included linoleoyl chloride (34.62%), linoleic acid (33.21%), and 2-4-di-tert-butylphenol (5.79%). Hemp seed oil's ability to scavenge radicals was studied through the use of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 1,1-diphenyl-2-picrylhydrazil bioanalytical radical scavenging methods. The results unveiled its potent radical-scavenging properties, with an 46.20 µg/mL for 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radicals and IC_{50} of 9.76 µg/mL for 1,1-diphenyl-2-picrylhydrazil radicals. The investigation also extended to explore the reducing capabilities of Fe^{3+}-2,4,6-tri(2-pyridyl)-S-triazine, copper (Cu^{2+}), and iron (Fe^{3+}). Hemp seed oil demonstrated notable inhibitory effect against α-amylase (IC_{50}: 545.66 µg/mL), acethylcholinesterase (IC_{50}: 28.00 µg/mL), and carbonic anhydrase II (IC_{50}: 322.62 µg/mL).

Conclusions This interdisciplinary research will prove valuable and set the stage for future investigations into the antioxidant characteristics and enzyme inhibition patterns of plants and plants oils that hold medical and industrial significance.

Keywords *Cannabis sativa*, Hemp seed oil, Chromatography, Antioxidant activity, Enzyme inhibition, LC–HRMS

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Background

Today, herbs have emerged as promising resources for developing new products within the pharmaceutical, food, and cosmetic industries. The pharmaceutical industry highly regards medicinal and aromatic plants for their abundant chemical components, encompassing active substances like flavonoids, polyphenols, alkaloids, glycosides, and tannins, which can be utilized in drug synthesis. Beyond their medical applications, these plants also hold significant value for their rich nutrition content and active substances, including vitamins and essential oil components [1, 2]. The pharmacological effects of medicinal herbs are primarily linked to their secondary metabolites, which are smaller molecules in contrast to the primary metabolites such as carbohydrates, lipids, and proteins. Plant secondary metabolites serve a critical role in the defense and survival mechanisms of plants, without being directly involved in their regular growth or reproductive processes. These compounds serve as major sources of food additives such as antioxidants, antibiotics, and pesticides. Many biosynthesized compounds originating from secondary metabolites, including phenols, alkaloids, steroids, essential oils, tannins, and lignins, have demonstrated great potential as drug leads. Their diverse and beneficial properties make them promising candidates for developing new medicines and enhancing various human applications [3].

Reactive oxygen species (ROS) can be categorized as either radicals, which include at least one unpaired electron, or as non-radicalic reactive compounds with the capacity to oxidize biomolecules. Consequently, these intermediate compounds are also referred to as pro-oxidants or oxidants. Oxidative stress had a crucial role in onset of numerous diseases, including neurodegenerative and cardiovascular disorders, aging process as well as the cancer [4]. To counteract oxidative stress, the body relies on an antioxidation system comprising enzymatic and non-enzymatic antioxidants. This system works together to defend against the harmful effects of oxidative stress [5]. Based on multiple epidemiological, clinical, and in vitro studies, it has been demonstrated that phenolic compounds possess antioxidant properties, thereby diminishing the likelihood of various degenerative diseases such as neurodegenerative diseases, cancer, osteoporosis, cardiovascular diseases, and diabetes. Additionally, phenolic compounds exhibit a several biological activities [6]. Currently, a wide range of synthetic antioxidants are commonly utilized. However, their usage has faced limitations due to concerns regarding potential toxicity and carcinogenicity, leading to regulatory restrictions. Hence, there is a rising curiosity in the exploration of plant-based antioxidants from plant-based sources that can effectively combat free radical damage. Moreover, the extent of antioxidant activity is influenced by both the quantity and the arrangement of hydroxyl groups (-OH) on the aromatics. It is widely accepted that the capacity to act as a hydrogen donor and inhibit oxidation is augmented with an increase in the count of -OH groups present in the phenol ring. Consequently, the presence of more hydroxyl groups on the aromatic ring enhances the antioxidant properties of a compound [7].

Cannabis sativa, a one-year herbaceous plant from the Cannabinaceae family, has its origins in Central Asia and has a profound historical significance in the cultivation of food, fiber, and medicinal products. Hemp seeds are rich in various bioactive phytochemicals, including cannabinoids, tocopherols, terpenes, polyphenols, and microminerals [8]. Hemp seeds are comprised of more than 30% oil, with over 80% of that oil consisting of multiple unsaturated fatty acids, specifically α-linolenic acids and linoleic. These fatty acids, particularly ω-3, are associated with a range of benefits, such as anti-inflammatory, anticancer, and antithrombotic properties. They are also known to stimulate overall metabolism and promote fat burning. The ω-6/ω-3 ratio in hemp seed oil is roughly 3:1, a ratio that is regarded as optimal for human health. Recently, some research has brought to light the valuable impacts of these fatty acids, as well as the consumption of hemp seeds, on different aspects of cardiovascular well-being, encompassing platelet aggregation and ischemic heart disease. Additionally, hemp seeds' oil and their contents have gained recognition as valuable, antioxidant-rich foods owing to the existence of bioactive molecules. However, additional research and clinical trials are essential to evaluate any possible adverse effects of hemp products in dietary use [9].

Alzheimer's disorder (AD) is categorized as a neurodegenerative disease, and projections suggest that by 2050, it will affect more than 100 million individuals, contributing to a total of approximately 150 million individuals affected by all types of dementia [10]. While important progress has been made in our comprehension of AD pathogenesis and the evolution of its conceptualization since Alois Alzheimer documented the first case in 1907, it remains a challenge that there are currently no disease-modifying treatments available [11]. Within AD patients' brain tissue, acetylcholinesterase (AChE) is found in higher abundance when compared to butyrylcholinesterase (BChE), and this excess presence plays a role in the degradation of acetylcholine (ACh) in the hippocampus and cerebral cortex. Cholinesterase inhibitors (ChEIs), such as AChE and BChE inhibitors, work by preventing the breakdown of the neurotransmitter, leading to increased levels of brain ACh, thereby increasing the impaired cholinergic neurotransmission in the brain [12].

One of the most effective approaches to prevent hyperglycemia and diabetes involves regulating blood glucose levels. The sugar molecules found in the bloodstream result from the hydrolysis of carbohydrate molecules, a process catalyzed by certain digestive enzymes, such as α-glycosidase [13]. This enzyme, α-glycosidase, is located in the intestinal membrane and is responsible for the hydrolysis of polysaccharide molecules. Additionally, the aldose reductase facilitates the transformation of glucose molecules into sorbitol, serving a vital function in the development of diabetic complications in various parts of the human body. Hence, digestive enzyme inhibitors derived from natural sources have the potential to be employed in the managing hyperglycemia and type-2 diabetes mellitus (T2DM) [14].

Glaucoma is a common eye disease that, when not detected and treated, can result in permanent blindness. Typically, the initial treatment for glaucoma involves the application of topical medications, including selective or non-selective β-blockers or topical prostaglandin analogs. If these first-line treatments prove insufficient, α-agonists and topical carbonic anhydrase (CA) inhibitors are often selected as second-line options. Parasympathomimetic agents, with pilocarpine being the most common, are considered third-line choices for treatment [15]. Human carbonic anhydrases (hCAs), which are zinc metalloproteins, are well known for their ability to facilitate the conversion of carbon dioxide (CO_2) into protons (H^+) and bicarbonate ions (HCO_3^-) [16]. These enzymes play a pivotal role in various physiological processes, including CO_2 and pH balance maintenance, respiration, the exchange of CO_2 and HCO_3^- between the lungs and metabolizing tissues, participation in biosynthetic reactions, electrolyte excretion across different tissues and organs, implications in tumorigenicity, calcification and regulation of bone resorption, and numerous other pathological and physiological processes [17]. The primary mode of action for the majority of antiglaucoma medications involves the inhibition of hCA isoenzymes, particularly hCA I and hCA II isoforms. This inhibition results in lowered bicarbonate levels, thereby reducing the production of aqueous humor and alleviating elevated intraocular pressure. Among these isozymes, hCA II stands out as a crucial and highly effective regulator of intraocular pressure (IOP) [18].

When a detailed study is done in the literature, there are many studies done on hemp oil. It has been reported that cannabis has been used in different ways by humans since ancient times and this plant has various medical applications around the world. Up to now, some medical and cosmetic benefits of hemp oil discovered in the management of depression, inflammation, chronic pain, hair and skin care, vomiting, nausea, multiple sclerosis, cancer, and hepatitis [19, 20]. In some studies, it is observed that hemp oil has many biological effects such as toxicological and antinociceptive. However, there is no study that studies the combined antioxidant, antiglaucoma, antidiabetes, and anti-Alzheimer's properties of hemp oil [21]. In this study, we evaluated the hemp seed oil chemical composition, investigating its potential antioxidant properties, its impact on Alzheimer's disease (AD), and its effectiveness in managing diabetes. Furthermore, we employed advanced analytical techniques, GC–MS/FID and LC–HRMS, to pinpoint the presence of polyphenols and essential oils in hemp seed oil.

Materials and procedures

Chemicals

Trolox, 2,9-dimethyl-1,10-phenanthroline (neocuproine), butylated hydroxytoluene (BHT), α-tocopherol 2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), butylated hydroxyanisole (BHA), and 1,1-diphenyl-2-picryl-hydrazyl (DPPH¨) were provided from Sigma-Aldrich Chemie GmbH, and fumaric acid, ascorbic acid, caffeic acid, chlorogenic acid, vanillic acid, naringin, rutin, p-coumaric acid, syringic acid, salicylic acid, rosmarinic acid, naringenin, quercetin, chrysin, luteolin, and emodin were supplied from Sigma-Aldrich. Hyperoside, luteolin 7-glycoside, (+)-trans-taxifolin, orientin, apigenin, hispidulin, acacetin, hederagenin, and acacetin were supplied from TRC in Canada. Luteolin-7-rutinoside and verbascoside were obtained from HWI Analytik GMBH and Carbosynth companies, respectively. Hesperidin was obtained from J&K Company. Myricetin was obtained from Carl Roth GmbH & Co. Penduletin, isosakuranetin, and dihydrokaempferol were provided from Phytolab. Apigenin 7-glycoside was obtained from the EDQM CS. Caffeic acid phenethylester and nepetin were acquired from the European Pharmacopoeia and Supelco, respectively.

Preparation of hemp (*Cannabis* sativa) seed oil

Hemp (*C. sativa*) seeds were acquired from a domestic market. The steam distillation method has been employed to extract hemp seed oil. This process comprises several continuous distillation stages utilizing steam as the stripping gas to separate the oils. By directly applying steam to the plant, a vapor combination is generated, which is later condensed to yield a liquid with distinct oil and water layers. The upper layer consists of essential oil, which contains water-insoluble compounds, while the lower portion contains a hydrolysate or hydrosol with water-soluble components. Cohobation can be employed to recover polar compounds left in the water. The seeds were positioned on a grid situated above the vapor inlet of the vapor distillation unit. Vapor was

introduced into the unit for about two hours, and the resulting combination of water vapor and vaporized oils was compressed using a cooler and subsequently gathered in a designated container. The temperature of the cooler was set to 35 °C, and the cooling process was carried out for 45 min. The hemp seed oil, separated from the water due to its density difference, was then extracted from the collection container.

Analysis of polyphenol profiling with LC–HRMS

One of the most used techniques in the literature for the determination of phenolic compounds of natural resources is HPLC–MS technique. Our group has also done a lot of work on this subject. In this study, the LC–HRMS technique was chosen rather than the LC–MS/MS technique because it provides high mass resolution. Thus, we can have the opportunity to accurately and precisely identify and quantify multiple molecules that we have monitored at the same retention time, without the need for chromatographic separation due to high mass resolution. For this reason, the LC–HRMS analyses were conducted employing a Thermo Orbitrap Q-Exactive mass spectrometer, which is made by Thermo Fisher Scientific Inc. (Waltham, MA, USA). The system was outfitted with a Troyasil C18 column, measuring 150 × 3 mm i.d., and featuring a particle size of 3 µM. The experiments took place in Istanbul, Türkiye, as described in our previous study. Briefly, mobile phase A was formulated with 1% formic acid in water, and mobile phase B comprised 1% formic acid in methanol. The gradient program employed in the experiments was as follows: from 0 to 1.00 min, 50% A and 50% B; from 1.01 to 6.00 min, 100% B; and finally, from 6.01 to 10 min, 50% A and 50% B [22]. The mobile phase was kept at a flow rate of 0.35 mL/min, and the column temperature was adjust to 22 °C. The surrounding conditions were controlled with a temperature set at 22.0 ± 5.0 °C and a relative humidity maintained at 50 ± 15% [23]. The samples (1.4 mL) were added to capped auto-sampler vials from which samples (5 µL) were injected into the LC for analysis. In order to measure the components present at very low concentration in the extract within the detection limits of the compounds, the injection volume was repeated as 50 µL instead of 5 µL. Samples were stored in the auto-sampler at 15 °C. Based on previous experiences and literature data, it was established that an acidified methyl alcohol and water gradient is the optimal solvent system for achieving favorable ionization plentiful and efficient compound separation in HPLC. Given that the electrospray ionization (ESI) source is known to offer excellent ionization efficiency for small and moderately polar substances, the ESI source was selected for the presented method ın this study. The instrument's high-resolution mode was used to scan ions within the m/z range of 85 to 1500. The process of identifying compounds was accomplished by analyzing their HRMS data and retention time with those of standards, with purity ranging from 95 to 99% (as described in Sect. 2.1). Dihydrocapsaicin, with a purity of 95%, served as an internal standard for the LC–HRMS measurements. The use of an internal standard helped mitigate repeatability issues arising from external factors, such as ionization repeatability, in the MS measurements. In Table 1, the mass parameters for each specified compound can be found. A detailed account of the LC–HRMS method, the experiment for uncertainty analysis, and the confirmation parameters for phenolics have been previously supplied in comprehensive detail.

The verification of the LC–HRMS method was accomplished by employing analytical standards of the specified compounds, utilizing either positive or negative ions, as specified in Table 1. Dihydrocapsaicin served as the internal standard in the validation process. The method validation parameters included linearity, selectivity, recovery, intermediate precision, repeatability, limit of detection (LOD), and limit of quantification (LOQ). The determination of LODs for individual chemicals in the method has been carried out using the following formula: LOQ or LOD = κSDa/b, where LOQ is 3 and κ is 3 for LOD. In this context, SDa denotes the standard deviation of the intercept, while "b" signifies the slope. For detailed information on the validation procedure, as well as the methodology for uncertainty appraisal of the operative method, please refer to our previously published paper [24].

Isolation and analysis of essential oil of hemp seed oil with GC/MS and GC–FID

Following the drying of the extract with dehydrated $CaCl_2$, the essential oil was kept at 4 °C until it was ready for GC–MS/FID analysis. The oil yield was determined as 1.52%. GC–MS analysis was performed using a Thermo Scientific Trace GC 1310 coupled to a Thermo TSQ 9610 MS system. A DB-5 capillary column with dimensions of 60 m × 0.25 mm and a 0.25 mm of thickness of film was employed. Helium was used as the transporter gas at a flow rate of 0.8 mL/min. The GC oven temperature was initially set at 80 °C for 10 min and then programmed to increase at a rate of 4 °C/min until it reached 280 °C, where it was held constant for 5 min. A split ratio of 1:20 was used, and the injector temperature was maintained at 250 °C. Mass spectra were obtained at 70 eV within the mass range of m/z 35–650. For GC–FID analysis, a Thermo Scientific Trace GC 1310 instrument was employed, and the FID detector temperature was configured at 280 °C. To ensure the same elution order as observed in GC–MS analysis, simultaneous

Table 1 Composition of chemicals and the validation parameters of hemp (*Cannabis sativa*) seed's oil (mg/L oil) determined by LC–HRMS

Phenolic compounds	Molecular formula	m/z	Ionization mode	Linear range	Linear regression equation	LOD/LOQ	R^2	Recovery	Phenolics	U%
Ascorbic acid	$C_6H_8O_6$	175.0248	Negative	0.5–10	$y=0.00347x-0.00137$	0.39/1.29	0.9988	96.20	4.21	3.94
Epigallocatechin	$C_{15}H_{14}O_7$	307.0812	Positive	0.3–5.0	$y=0.00317x+0.000443$	0.17/0.57	0.9947	102.22	0.61	3.09
Chlorogenic acid	$C_{16}H_{18}O_9$	353.0878	Negative	0.05–10	$y=0.00817x+0.000163$	0.02/0.06	0.9994	96.68	0.08	3.58
Fumaric acid	$C_4H_4O_4$	115.0037	Negative	0.1–10	$y=0.00061x-0.0000329$	0.05/0.17	0.9991	97.13	–	2.88
Verbascoside	$C_{29}H_{36}O_{15}$	623.1981	Negative	0.1–10	$y=0.00758x+0.000563$	0.03/0.1	0.9995	96.19	–	2.93
Orientin	$C_{21}H_{20}O_{11}$	447.0933	Negative	0.1–10	$y=0.00757x+0.000347$	0.01/0.03	0.9993	96.22	–	3.67
Caffeic acid	$C_9H_8O_4$	179.0350	Negative	0.3–10	$y=0.0304x+0.00366$	0.08/0.27	0.9993	94.51	–	3.74
Luteolin-7-rutinoside	$C_{27}H_{30}O_{15}$	593.1512	Negative	0.1–10	$y=0.00879x+0.000739$	0.01/0.03	0.9988	93.05	0.03	3.06
Luteolin-7-glycoside	$C_{21}H_{20}O_{11}$	447.0933	Negative	0.1–7.0	$y=0.0162x+0.00226$	0.01/0.03	0.9961	96.31	–	4.14
Rutin	$C_{27}H_{30}O_{16}$	609.1461	Negative	0.05–10	$y=0.00329x-0.00005576$	0.01/0.03	0.999	96.97	0.49	3.07
Rosmarinic acid	$C_{18}H_{16}O_8$	359.0772	Negative	0.05–10	$y=0.00717x-0.0003067$	0.01/0.03	0.9992	99.85	–	3.77
Hyperoside	$C_{21}H_{20}O_{12}$	463.0882	Negative	0.05–10	$y=0.0072x-0.00003096$	0.01/0.03	0.9995	96.62	–	3.46
Apigenin 7-glycoside	$C_{21}H_{20}O_{10}$	431.0984	Negative	0.3–7.0	$y=0.0246x+0.00306$	0.01/0.03	0.9962	96.07	–	2.86
Ellagic acid	$C_{14}H_6O_8$	300.9990	Negative	0.05–10	$y=0.0085x-0.000612$	0.03/1	0.9994	101.49	–	3.59
Quercitrin	$C_{21}H_{20}O_{11}$	447.0933	Negative	0.05–10	$y=0.0179+0.0003331$	0.01/0.03	0.999	97.00	–	4.20
Quercetin	$C_{15}H_{10}O_7$	301.0354	Negative	0.1–10	$y=0.0509x+0.00467$	0.01/0.03	0.9978	96.41	–	3.78
Herniarin	$C_{10}H_8O_3$	177.0546	Positive	0.1–7.0	$y=0.309x+0.0266$	0.01/0.03	0.9983	92.92	–	2.95
Salicylic acid	$C_7H_6O_3$	137.0244	Negative	0.3–10	$y=0.0361x+0.00245$	0.01/0.03	0.9982	92.88	0.85	3.89
Naringenin	$C_{15}H_{12}O_5$	271.0612	Negative	0.1–10	$y=0.0281x+0.00182$	0.01/0.03	0.9995	86.65	0.08	1.89
Luteolin	$C_{15}H_{10}O_6$	285.0405	Negative	0.1–10	$y=0.117x+0.00848$	0.01/0.03	0.9981	96.98	0.11	4.20
Apigenin	$C_{15}H_{10}O_5$	269.0456	Negative	0.3–10	$y=0.104x+0.0199$	0.01/0.03	0.9998	81.55	14.60	3.42
Hispidulin	$C_{16}H_{12}O_6$	301.0707	Positive	0.05–10	$y=0.02614x+0.0003114$	0.01/0.03	0.9993	98.36	0.03	2.87
Isosakuranetin	$C_{16}H_{14}O_5$	285.0769	Negative	0.05–10	$y=0.0235x+0.000561$	0.01/0.03	0.9992	96.56	–	3.41
Penduletin	$C_{18}H_{16}O_7$	343.0823	Negative	0.3–10	$y=0.0258x+0.00253$	0.01/0.03	0.9991	83.43	0.70	3.20
CAPE	$C_{17}H_{16}O_4$	283.0976	Negative	0.3–7.0	$y=0.255x+0.0477$	0.01/0.03	0.9964	94.42	–	3.13
Chrysin	$C_{15}H_{10}O_4$	253.0506	Negative	0.05–7.0	$y=0.0964x-0.0002622$	0.01/0.03	0.999	87.92	–	3.24
Quillaic acid	$C_{30}H_{46}O_5$	485.3273	Negative	0.05–10	$y=0.00781x-0.0001318$	0.01/0.03	0.9992	90.29	4.21	2.56
Caryophyllene oxide	$C_{15}H_{24}O$	221.1900	Positive	3.0–7.0	$y=0.00151x+0.00692$		0.9909	96.87	0.61	4.05

auto-injection was carried out in duplicate on the same column under identical operational conditions. The relative percentage of the separated compounds was determined from the FID chromatograms [25]. Kovats Indices (KI) were calculated using alkanes as reference points. Compound identification was achieved by analyzing their retention times and mass spectra with those sourced from genuine samples, as well as utilizing the Wiley spectra and NIST and relevant literature data [26].

Assays for measuring reducing ability

To determine the Fe^{3+} reduction potential of hemp seed oil, the $Fe^{3+}(CN^{-})_6$ complex reducing method was employed [27]. The procedure involved transferring various concentrations of hemp seed oil to test tubes, followed by the addition of 2.5 mL of buffer mixture (pH 6.6, 0.2 M) and 2.5 mL of a 1% [$K_3Fe(CN)_6$] solution. The mixture was vortexed and then incubated at 50 °C for 25 min. Subsequently, 2.5 mL of 10% TCA was added. A portion of the upper layer (2.5 mL) from the solutions was blended with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution. The spectrophotometric absorbance of the preventive effects of hemp seed oil was documented at 700 nm.

After completing all the required experimental processes, the absorbance of the Cu^{2+} reducing power of hemp seed oil was recorded based on a previously conducted study [28]. The experimental procedure involved transferring 0.25 mL of $CuCl_2$ (10 mM), 0.25 mL of ethanolic neocuproine (7.5×10^{-3} M), and 250 µL of NH_4Ac buffer (1.0 M) into test tubes. Additionally, test tubes containing 10, 20 and 30 µg/mL of hemp seed oil were prepared. Final volume was then adjusted to 2 mL using distilled water, and the absorbance values of the mixtures were recorded at 450 nm after a 30-min incubation period.

In the assessment of the Fe^{3+}-TPTZ complex reducing ability of hemp seed oil, the experimental procedure was conducted based on a previous study [29]. The procedure involved preparing a fresh solution of TPTZ. This was done by dissolving 2.25 mL of TPTZ in a 10 mM concentration using 40 mM HCl. The prepared TPTZ solution was then transferred to a test tube containing 2.5 mL of acetate buffer (0.3 M, pH 3.6) and 2.25 mL of $FeCl_3$ solution (20 mM). After that, different concentrations of hemp seed oil were transferred to the mixture and incubated at 37 °C for a duration of 25 min. Finally, the absorbance of the reducing power of hemp seed oil was measured spectrophotometrically at 593 nm [30]. To ensure accuracy and reliability, all experiments related to the assessment of reducing abilities were repeated three times. The outcomes achieved from these repetitions were then averaged to calculate the arithmetic mean [31].

Assessment of radical scavenging activities

The radical scavenging capacity of hemp seed oil was assessed using the $DPPH^{\cdot}$ scavenging assay, following the procedure outlined by Blois [32]. First, 1 mL of $DPPH^{\cdot}$ solution with a concentration of 0.1 mM, which was prepared in ethanol and possessed a blue color, was added to hemp seed oil samples at various concentrations (ranging from 10 to 30 µg/mL). Then the mixture was incubated at 25 °C for a period of 25 min and the absorbance values of the samples were recorded at a wavelength of 517 nm using a spectrophotometer. The $ABTS^{\cdot+}$ scavenging ability of hemp seed oil was assessed using Gulcin's method [33]. Firstly, an aqueous solution of ABTS with a concentration of 7.0 mM is prepared. To generate the $ABTS^{\cdot+}$ radical cation, oxidants such as $K_2S_2O_8$ are added to the ABTS solution at a concentration of 2.5 mM. Prior to use, the $ABTS^{\cdot+}$ solution underwent dilution with a buffer solution (0.1 M, pH 7.4). The absorbance value of the control was adjusted to 0.750 ± 0.025 at 734 nm for accurate measurements. Subsequently, different concentrations (10–30 µg/mL) of hemp seed oil were mixed with 1 mL of $ABTS^{\cdot+}$ solution in a 3 mL volume. The absorbance of the remaining $ABTS^{\cdot+}$ solution was quantified at 734 nm after a 30 min incubation period [34].

The radical scavenging content (RSC) of hemp seed oil was determined using the following formula: RSC (%) = (1 − Ac/As) × 100, where Ac represents the absorbance value of the control and As represents the absorbance value of the sample. Additionally, the IC_{50} value was obtained from the graphs and expressed as µg/mL [35].

Assay for inhibiting acetylcholinesterase activity

The evaluation of hemp seed oil's inhibitory effects on AChE was conducted following the methods outlined in our previous studies [36]. The assay utilized acetylthiocholine iodide (AChI) and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) as substrates. In a test tube, a mixture was prepared by combining 1 mL of Tris/HCl buffer (1.0 M, pH 8.0), 10 µL of hemp seed oil at varying concentrations, and 50 µL of AChE. Then, the sample was incubated at 25 °C for fifteen minutes. Subsequently, 50 µL of DTNB (0.5 mM) was added, followed by the addition of 50 µL of AChI (10 mM) to initiate the reaction. The absorbance was then measured at 412 nm.

Assay for inhibiting α-amylase activity

The inhibitory effect of hemp seed oil on α-amylase was assessed by employing a starch as substrate, following the protocol by Xiao [37] as given previously [38]. The procedure began by dissolving 1 g of starch in 50 mL of NaOH (0.4 M) and heating it at 75 °C for 20 min. Following the cooling process, the solution's pH was regulated to 6.9, and the volume was adjusted to 100 mL with deionized

water. Next, a mixture was prepared by combining 35 µL of the starch solution, 35 µL of phosphate buffer (pH 6.9), and 5 µL of the hemp seed oil solutions. The mixture was then left at 37 °C for 20 min. Following the initial incubation, 20 µL of α-amylase solution was added to the mixture and further incubated for an additional 20 min. To halt the reaction, 50 µL of 0.1 M HCl was introduced, and the absorbance of the resulting solution was gauged at 580 nm.

hCA II purification by affinity chromatoghraphy and inhibition assay

CA II, which catalyzes the interconversion between CO_2 and water and the dissociated ions of H_2CO_3, was sourced from human erythrocytes [39]. High purity of hCA II was obtained through the utilization of the sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatoghraphy [40]. The protein content was assessed using the Bradford's method at each stage of the purification assay by using BSA as the standard protein [41]. SDS-PAGE was employed to evaluate the purity of hCA II, following the methodology outlined in our previous research [42]. Additionally, during the purification procedure and inhibition studies of hCA II, esterase activity was quantified spectrophotometrically at 348 nm [43].

Determination of IC_{50} value

The inhibitory ability of hemp seed oil was determined by calculating IC_{50} values. The IC_{50} values were obtained by analyzing the graphs derived from the enzyme activity measurements, which correlated with increasing concentrations of hemp seed oil [44].

Statistical analysis

Data were analyzed using Student's *t*-test, and the software used was GraphPad Prism 6 (GraphPad, La Jolla, CA, USA, Software 7.0). Data are presented as mean ± standard deviation (SD). The minimum significance level was determined as $p < 0.05$. The statistical analyses we used in our study are extremely important in terms of producing meaningful results while evaluating our scientific research data. Within the scope of statistical analysis applications, Student's t-test was used to follow a systematic path and to ensure that we complete our research effectively.

Results

The LC–HRMS method was confirmed by assessing several parameters including linearity, precision, selectivity, accuracy, matrix effect, recovery, and analytes' stability. Within this work, 13 phenolic compounds were provisionally recognized from hemp seed oil. Based on the LC–HRMS results (Figure 1 and Table 1), hemp seed oil had abundant in terms of apigenin (14.60 mg/L oil), quillaic acid (4.21 mg/L oil), and ascorbic acid (4.21 mg/L oil). The species' secondary metabolite composition was determined by extracting the hemp seed oil using the liquid–liquid extraction method. To prepare the hemp seed oil sample for analysis, 100 mg of the oil was dissolved in a 4 mL volumetric flask using mobile phase B (1% formic acid in methyl alcohol) and subjected to ultrasonication for 10 min. Next, 100 µL of the internal standard (dihydrocapsaicin solution in methyl alcohol) was added, and the volume was adjusted using mobile phase B. The resulting solution was then filtered through a 0.45 µm Millipore Millex-HV filter. Subsequently, to prepare for each run, 1 mL of the final solution was added into an auto-sampler vial with a sealing cap, and then, 2 µL of the sample was loaded into the LC system. The samples in the auto-ampler were maintained at a temperature of 15 °C throughout the experimental studies.

GC–MS was employed to conduct precise qualitative analysis of the constituents of various essential oils and aromatic compounds [45]. Table 2 provides relative information regarding the aromatic components found in hemp seed oil. In this study, a total of 6 volatile compounds were identified in the hemp seed oil samples. Among these, linoleoyl chloride (34.62%), linoleic acid (33.21%), 2-4-di-tert-butylphenol (5.79%), palmitic acid (4.83%), stenol (3.74%), and cetal (3.19%) were the most prevalent components in hemp seed oil (Figure 2 and Table 2).

Hemp (*C. sativa*) seed's oil displayed notable reducing capability in $Fe[Fe(CN^-)_6]_3$, Fe^{3+}-TPTZ, and Cu^{2+} reducing assays [46]. Initially, a conversion assay was conducted to evaluate the reduction potential of hemp seed oil by measuring the interconversion between Fe^{3+} and Fe^{2+} (see Fig. 3A and Table 3). In the following order, at 50 µg/mL, both hemp seed oil and standards ($r^2 = 0.9804$) demonstrated Fe^{3+} reductive capability ($p < 0.01$): Ascorbic acid (2.298 ± 0.086, $r^2 = 0.9659$) ≥ BHA (2.292 ± 0.012, $r^2 = 0.9993$) ≥ BHT (2.136 ± 0.090, $r^2 = 0.9957$) > Trolox (1.514 ± 0.066, $r^2 = 0.9963$) > α-tocopherol (0.862 ± 0.038, $r^2 = 0.9996$) ≥ hemp seed oil (0.636 ± 0.009, $r^2 = 0.9830$). The rise in absorbance indicates the complex formation and an enhanced capacity for reduction (Fig. 3A). Furthermore, the Fe^{3+}-TPTZ and Cu^{2+} reducing abilities of hemp seed oil were examined, and their results are presented in Fig. 3B, C and Table 3. Hemp seed's oil exhibited good absorbance values at the various concentrations. In the following order, hemp seed's oil and standards at 30 µg/mL demonstrated a reduction of Cu^{2+} ions (Fig. 3B): BHA (2.418 ± 0.018, $r^2 = 0.9887$) > BHT (1.953 ± 0.045, $r^2 = 0.9998$) > Trolox (1.800 ± 0.096, $r^2 = 0.9974$) > hemp seed oil (1.208 ± 0.061, $r^2 = 0.9655$) > ascorbic acid (0.983 ± 0.048,

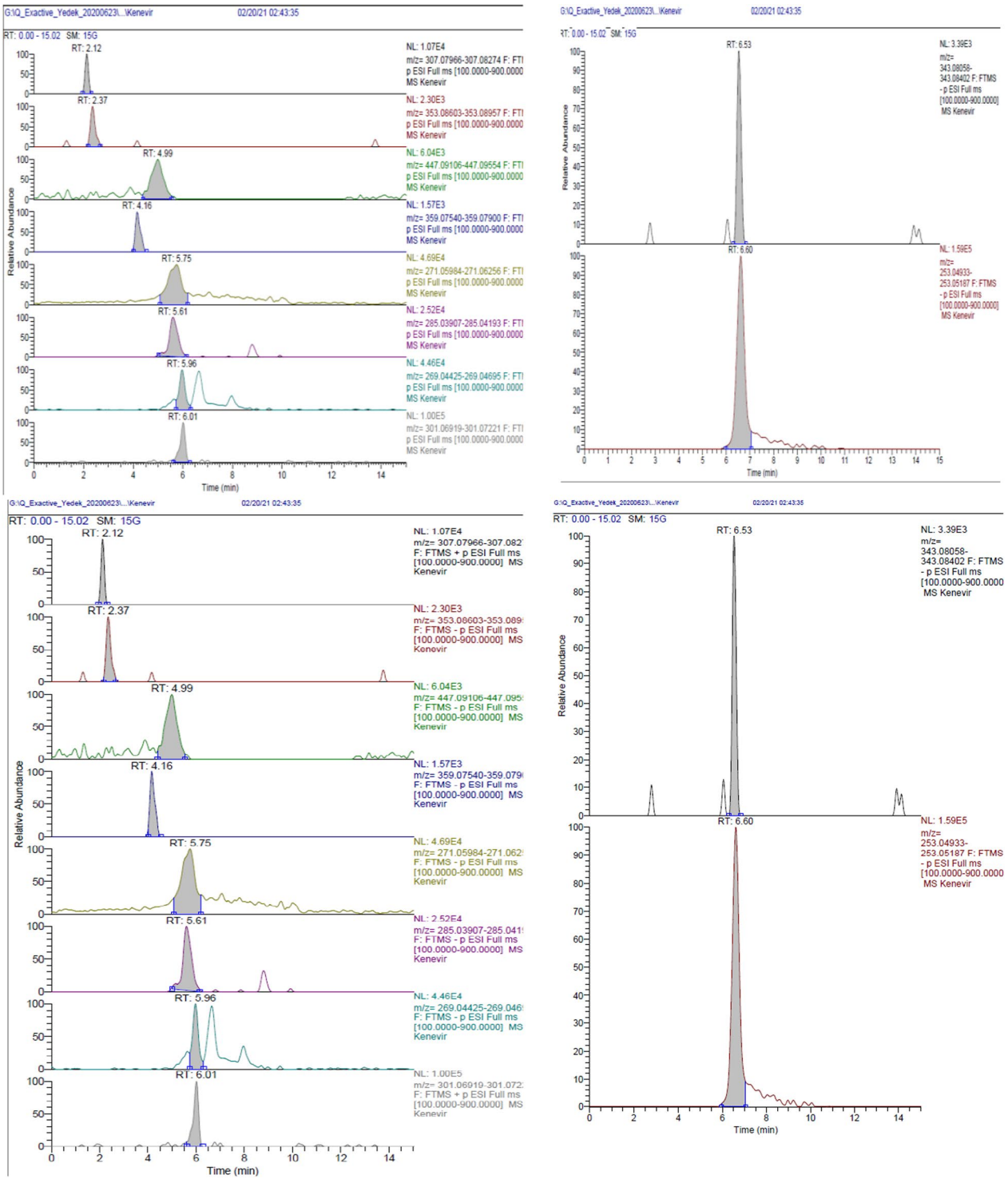

Fig. 1 LC–HR/MS chromatograms of hemp (*Cannabis sativa*) seed's oil

$r^2 = 0.9822$) > α-tocopherol (0.851 ± 0.046, $r^2 = 0.9994$). In this reduction assay, hemp seed oil from hemp demonstrated a notable reducing capacity (Fig. 3C and Table 3). The FRAP reducing capacity of the test materials, including hemp's seed oil and standards, decreased in the subsequent sequence: Ascorbic acid (1.257 ± 0.024, $r^2 = 0.9869$) > Trolox (1.180 ± 0.032, $r^2 = 0.9732$) ≥ BHA (1.172 ± 0.014, $r^2 = 0.9605$) > α-tocopherol (0.918 ± 0.011,

Table 2 Chemical components of the essential oil provided from hemp (*Cannabis sativa*) seed's oil analyzed via GC–MS

Essential oils	Formula	RT	Contents (%)
2-4-Di-tert-butylphenol	$C_{14}H_{22}O$	14.63	5.79
Cetal	$C_{17}H_{34}O$	16.15	3.19
Stenol	$C_{18}H_{38}O$	19.83	3.74
Linoleic acid	$C_{18}H_{32}O_2$	25.37	33.21
Palmitic acid	$C_{16}H_{32}O_2$	22.65	4.83
Linoleoyl chloride	$C_{18}H_{31}ClO$	25.45	34.62
Total			85.38

$r^2 = 0.9904$) > BHT (0.690 ± 0.008, $r^2 = 0.9645$) > hemp seed's oil (0.515 ± 0.009, $r^2 = 0.9604$).

The observed IC_{50} for the DPPH radical scavenging abilities of hemp (*C. sativa*) seed's oil and standards followed the subsequent order: 5.82 µg/mL for ascorbic acid ($r^2 = 0.9668$) < 6.03 µg/mL for Trolox ($r^2 = 0.9925$) < 6.86 µg/mL for BHA ($r^2 = 0.9949$) < 7.70 µg/mL for α-tocopherol ($r^2 = 0.9961$) < 9.76 µg/mL for hemp seed's oil ($r^2 = 0.9982$) < 49.50 µg/mL for BHT ($r^2 = 0.9957$). The observed low EC_{50} values suggest that the tested samples exhibit strong and effective $DPPH^{\cdot}$ radical scavenging abilities (see Figure 4A and Table 4).

Figure 4B illustrates the concentration-dependent ABTS radical scavenging ability of hemp (*C. sativa*) seed's oil, indicating an effect at concentrations between 10 and 20 µg/mL ($p < 0.001$). In the $ABTS^{\cdot+}$ removal assay, hemp seed oil demonstrated an IC_{50} of 46.20 µg/mL ($r^2 = 0.9962$) (see Table 4). The EC_{50} values have been assessed for standard molecules: 6.35 µg/mL for BHA ($r^2 = 0.9746$) < 11.74 µg/mL, for ascorbic acid ($r^2 = 0.9983$) < 12.60 µg/mL for BHT ($r^2 = 0.9995$) < 16.50 µg/mL for Trolox ($r^2 = 0.9775$) < 18.72 µg/mL for α-tocopherol ($r^2 = 0.9347$) (Figure 4B).

Table 5 displays the inhibition outcomes for the related enzymes. Hemp (*C. sativa*) seed's oil inhibited AChE with IC_{50} of 28.00 µM ($r^2 = 0.9876$). An IC_{50} of 8.82 µM ($r^2 = 0.9836$) was obtained for the inhibition of AChE using the tacrine standard inhibitor, which was used for comparison. The moderate inhibitory effect of hemp seed's oil on α-amylase is evident from Table 4, with an IC_{50} value of 545.66 µM ($r^2 = 0.9356$). Hemp seed's oil demonstrated an IC_{50} of 322.62 µM ($r^2 = 0.9032$) against the cytosolic and predominant hCA II isozyme (Table 5). In comparison, acetazolamide (AZA), a clinical CA inhibitor, exhibited inhibition of the cytosolic and predominant hCA II isoforms with an IC_{50} amount of 9.96 µM ($r^2 = 0.9930$). Phenolic-rich plants are efficient at inhibiting CA. Cannabinoids, which are biologically active components, can be obtained from *Cannabis sativa* (phytocannabinoids) or synthesized chemically as pure compounds. Among the most extensively studied cannabinoids are CBD, which is the primary non-psychoactive compound found in cannabis, and plays a significant role in neurological and bioactive responses. Numerous studies indicate that CBD could offer a promising approach in alleviating corneal pain and inflammation.

Discussion

Throughout history, plants and their metabolites have been extensively employed to address various medical issues. Flavonoids, found abundantly in various plant parts, constitute the most prevalent and widely dispersed category of plant compounds. Within this group,

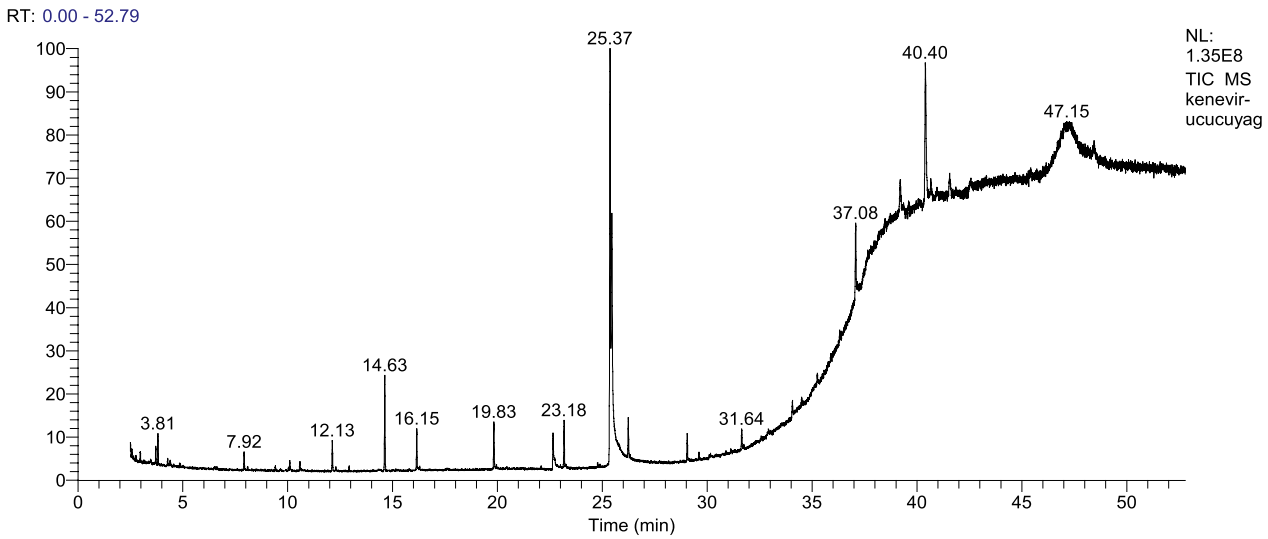

Fig. 2 GC/MS analysis of hemp (*Cannabis sativa*) seed's oil identified various compounds and their respective percentage ratios within the sample

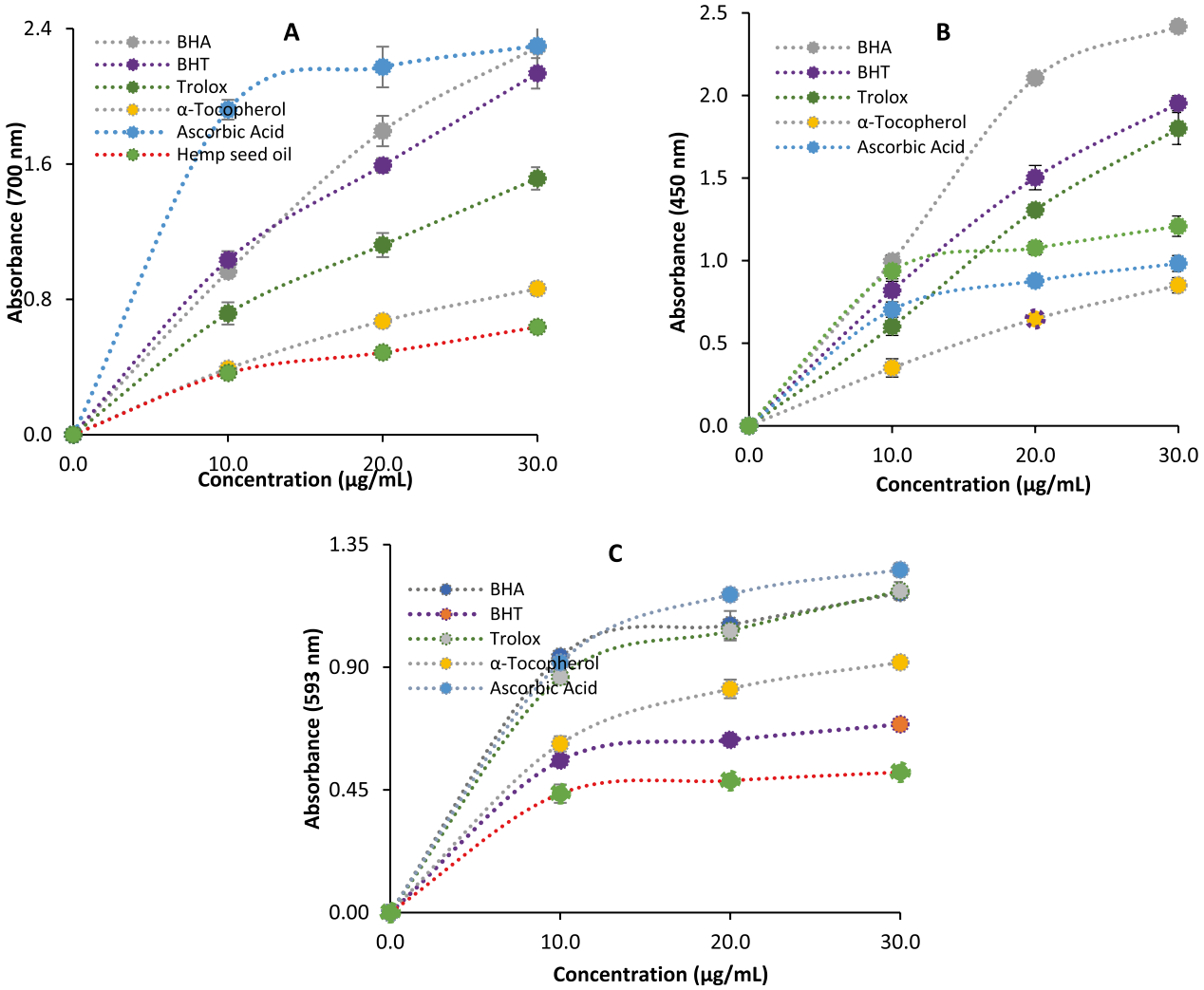

Fig. 3 Cupric (Cu^{2+}) ions, ferric (Fe^{3+}) ions, and Fe^{3+}-TPTZ complex decreasing potential of hemp (*Cannabis sativa*) seed's oil and standards

Table 3 The reduction potential of hemp (*Cannabis sativa*) seed's oil and standards at a concentration of 50 µg/mL was assessed for Fe^{3+}, Cu^{2+}, and Fe^{3+}-TPTZ (BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole)

Antioxidants	Fe^{3+} reducing*		Cu^{2+} reducing*		Fe^{3+}-TPTZ reducing*	
	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2
BHT	2.136 ± 0.090	0.9957	1.953 ± 0.045	0.9998	0.690 ± 0.008	0.9645
BHA	2.292 ± 0.012	0.9993	2.418 ± 0.018	0.9887	1.172 ± 0.014	0.9605
Trolox	1.514 ± 0.066	0.9963	1.800 ± 0.096	0.9974	1.180 ± 0.032	0.9732
Ascorbic acid	2.298 ± 0.086	0.9659	0.983 ± 0.048	0.9822	1.257 ± 0.024	0.9869
α-tocopherol	0.862 ± 0.038	0.9996	0.851 ± 0.046	0.9994	0.918 ± 0.011	0.9904
Hemp seed oil	0.636 ± 0.009	0.9830	1.208 ± 0.061	0.9655	0.515 ± 0.009	0.9604

*All values are displayed the mean ± standard deviation (SD) of three concurrent measurements ($n = 3$)

several subfamilies exist, including flavones, flavanols, flavanones, flavonols, and isoflavones. Their positive effects on health are closely linked to their antioxidant, anti-inflammatory, and anticancer properties [47]. Polyphenols, which are biologically active compounds, are abundantly present in plants and have demonstrated a

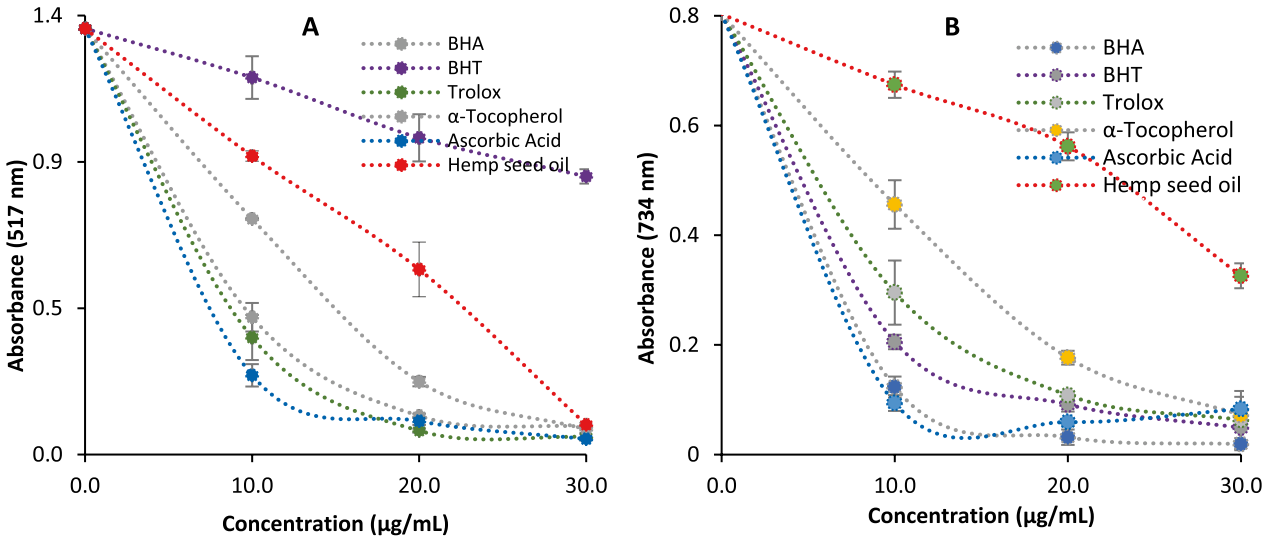

Fig. 4 Radical scavenging effects of hemp (*Cannabis sativa*) seed's oil and standards antioxidants using 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\cdot+}$) and 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) assays

Table 4 IC_{50} values (µg/mL) for the free radical scavenging activities of hemp (*Cannabis sativa*) seed's oil and standards using both 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\cdot+}$) and 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) assays

Antioxidants	$DPPH^{\cdot}$ scavenging		$ABTS^{\cdot+}$ scavenging	
	IC_{50}	r^2	IC_{50}	r^2
BHT	49.50	0.9957	12.60	0.9995
BHA	6.86	0.9949	6.35	0.9746
Trolox	6.03	0.9925	16.50	0.9775
Ascorbic acid	5.82	0.9668	11.74	0.9983
α-tocopherol	7.70	0.9961	18.72	0.9347
Hemp seed oil	9.76	0.9982	46.20	0.9962

Table 5 Half maximal concentration (IC_{50}; µg/mL) values of hemp (*Cannabis. sativa*) seed's oil against carbonic anhydrase II, acetylcholinesterase, and α-amylase enzymes

Enzymes	Hemp (*C. sativa*) seed oil		Standard inhibitors	
	IC_{50}	r^2	IC_{50}	r^2
Carbonic anhydrase II[a]	322.62	0.9032	9.96	0.9930
Acetylcholinesterase[b]	28.00	0.9876	8.82	0.9836
α-Amylase[c]	545.66	0.9356	7.54	0.9074

[a] Acetazolamide (AZA) was employed as the positive control for the carbonic anhydrase II isoenzyme

[b] Tacrine served as the positive control for butyrylcholinesterase and acetylcholinesterase enzymes

[c] Acarbose was utilized as the positive control for α-amylase enzyme

substantial protective effect against neural injuries and degeneration [48]. The declining effectiveness of synthetic drugs and the rising number of contraindications associated with their use have brought the utilization of natural medications back into the spotlight. There are several bioanalytical methods accessible for detecting and proving a correlation between phenolic substances and their uses. Given its abundant phenolic content, it is anticipated that hemp seed oil will exhibit substantial antioxidant activity.

In this study, the LC–HRMS method was validated by evaluating different parameters such as sensitivity, linearity, selectivity, matrix effect, accuracy, recovery, and stability of analytes [49]. Due to its exceptional sensitivity, LC–HRMS stands out as one of the best extensively employed chromatographic techniques for accurately quantifying polyphenols in phytoextracts [50]. The increasing prevalence of LC–HRMS, as evident from the growing number of publications utilizing the combination of LC and HRMS over the years, can be primarily attributed to recent advancements and the increased accessibility of more robust, sensitive, and selective instrumentation that operates at a lower cost. Hemp seed oil is particularly renowned for its abundance of polyunsaturated fatty acids. Additionally, it serves as a valuable antioxidant source like γ-tocopherol, which may have the potential to combat diseases linked to oxidative stress [51]. Cannabinoids, which are hydrophobic compounds originating from *Cannabis sativa*, possess different effects on the human body. They have been thoroughly investigated in cellular and animal models, and have also

undergone human clinical trials to assess their potential therapeutic benefits for a range of human conditions. These encompass conditions related to the central nervous system (CNS) and its malfunctions, such as certain forms of epilepsy, Parkinson's disease, multiple sclerosis, neuropsychiatric disorders, and pain management. Furthermore, the naturally occurring cannabinoid lipids known as endocannabinoids play a pivotal role in maintaining normal CNS function. If their regulation or modification can be controlled, they may offer an additional avenue for treating CNS diseases. Cannabinoids and endocannabinoids show potential as disease-modifying agents and therapeutic tools for preventing or addressing neurodegenerative diseases and neurological disorders [52].

The most prevalent phenolics in seed oil were found as apigenin, quillaic acid, and ascorbic acid. Apigenin (4′,5,7-trihydroxyflavone) is a natural flavonoid compound in different plants, including herbs, vegetables, and fruits. Apigenin was identified as the primary phenolics (14.60 mg/L oil) in hemp seed oil. Some potential health benefits and uses of apigenin include antioxidant properties, anti-inflammatory effects, cancer prevention, neuroprotection, anxiety-reducing, sedative effects, cardiovascular health, and antidiabetic properties [53]. Quillaic acid is a naturally occurring triterpenoid saponin compound found in the bark of the *Quillaja saponaria*. This compound has a variety of industrial and commercial uses, primarily due to its surfactant properties. Quillaic acid and its derivatives are used in a range of applications, including the food and beverage industry, cosmetics, pharmaceuticals, agriculture, personal care products, biotechnology, and vaccine production [54]. Ascorbic acid has a crucial role in acting as an antioxidant to safeguard cellular components against the detrimental effects of free radicals. It has been demonstrated that ascorbic acid effectively neutralizes free radicals within the watery environments found within cells and the circulatory system. Furthermore, it has been scientifically validated that ascorbic acid provides protection to cellular membranes and other hydrophobic compartments by regenerating the antioxidant state of ascorbic acid. Additionally, within these hydrophobic compartments, reduced Coenzyme Q collaborates with vitamin E to restore its antioxidant form [55].

The ferric ion reduction method is one of several assays used to measure antioxidant activity. It provides valuable information about plant-derived oils's ability to scavenge free radicals and alleviate oxidative stress. Under experimental conditions, when hemp seed oil is introduced into solutions containing Fe^{3+} ions, it results in the creation of a vivid blue $Fe_4[Fe(CN^{-})_6]_3$ complex, capable of light absorption at 700 nm [56]. Hemp seed's oil demonstrated an effective reducing capacity in Cu^{2+} reduction assays. This reductive effect was more than that of α-tocopherol and ascorbic acid. The results display that hemp seed's oil has a lower ability to reduce Fe^{3+}-TPTZ, which was almost equal to BHT and α-tocopherol, and also the reduction capacity in $Fe_4[Fe(CN^{-})_6]_3$ was almost equal to α-tocopherol. The CUPRAC technique is a commonly utilized analytical technique for assessing the antioxidant capacity of various compounds, including plant extracts [57]. When the relevant results are compared, it was observed that hemp seed oil was had strong Cu^{2+} reducing ability than that of ascorbic acid and α-tocopherol; however, this activity lover than BHA and BHT as synthetic and powerful antioxidants. In this investigation, the FRAP reduction assay was employed as the ultimate reduction assay. The FRAP assay quantifies antioxidant ability by assessing the reduction of ferric-tripyridyltriazine complex (Fe^{3+}-TPTZ) to the intense blue ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ) at a lower pH [39].

The $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ assays are commonly employed in research and the food industry to screen and compare the antioxidant effects of pure or natural compounds, extracts, and food products [58]. They help in understanding the potential health advantages of antioxidants by assessing their capability to counteract oxidative stress and reduce the risk of diseases associated with free radical damage, such as cardiovascular diseases and cancer. In a recent study, cannabidivarin and cannabidiol produced by *Cannabis sativa* exhibited notably lower activity compared to Trolox in scavenging ABTS and DPPH free radicals [59]. The hemp extracts demonstrated antioxidant effect toward in inhibiting the formation of DPPH radicals, with an IC_{50} was 60 µg/mL for the aqueous extract AE and 97 µg/mL for or the hexane extract HE [60]. Indeed, in our study, when hemp seed's oil was compared with standards that have strong DPPH radical scavenging properties, it was observed that hemp seed's oil was more effective than BHT and exhibited radical scavenging equivalent to BHA, α-tocopherol, and Trolox. Past research has shown a strong connection between antioxidant activity and polyphenols, which are known for their robust antioxidant properties. In these studies, a noteworthy correlation emerged between the antioxidant activity and phenolic compounds, as assessed using the DPPH method [61]. No prior research were found regarding apigenin glucuronide nor cannabidiol and Δ9-THC. The antioxidant activity is influenced not only by the total content of phenolic compounds but also by their specific characteristics, including their chemical structure, the position and number of -OH groups, and the nature of substitutions on the aromatic rings. These attributes play a crucial role in determining the overall antioxidant

effectiveness of these compounds [62]. Another interesting properties is that DPPH method is conventionally conducted under 50% ethanol/water, while ABTS assay is carry out in aqueous medium. In hemp seed's oil, the most phenolic compounds was measured. Apigenin that is sparingly soluble in aqueous buffers, so the scavenging ABTS free radicals is a little less than the other standard molecules.

Cannabis sativa derives its medicinal properties from a diverse range of secondary metabolites, which include terpenoids and flavonoids. Among these, cannabinoids are a unique class of terpenoids that specifically interact with mammalian endocannabinoid receptors. Consequently, extensive research has been dedicated to exploring the therapeutic effects of various cannabis compounds, particularly the cannabinoids Δ9-THC and CBD. These compounds have shown potential therapeutic benefits such as analgesia, psychoactivity, and antiviral properties [63]. ACh, a key neurotransmitter within the cholinergic system, assumes a significant role in the management of a wide array of psychiatric disorders [64]. The enzyme AChE acts to swiftly terminate neuronal impulse transmission by hydrolyzing acetylcholine in both the peripheral and CNS' cholinergic pathways. Consequently, AChE inhibitors have a long history of application to treat diverse neurological disorders, as well as for addressing issues related to memory and cognitive impairment [65]. *Cannabis sativa* is recognized for their high quantity of two significant terpenoids: Δ_9-THC and CBD. Both CBD and THC have been extensively studied for their central and peripheral activities, particularly in the context of various brain diseases such as AD and multiple sclerosis [66]. The psychoactive impact of cannabis has been ascribed to its primary constituent, Δ_9-THC, which is recognized as a powerful AChE inhibitor [67]. A research conducted by Sugarman and colleagues indicated that cannabinoids can lead to the inhibition of ACh release, thereby contributing to acute cognitive deficits. This effect is achieved by binding to presynaptic CB1-R receptors situated within cholinergic nerve terminals [68]. When taken together, these data showed that cannabinoids act on the cholinergic system, although an IC_{50} value was not reached for AChE inhibition. This is due to the significant influence of phytochemical composition on these types of bioassays.

Cannabinoids encompass some lipophilic compounds, which that synthesized by the human body as endocannabinoids, or found in the *Cannabis sativa* plant as phytocannabinoids. These compounds hold promise as an alternative to more aggressive treatments involving common corticosteroids, as they modified various biochemical pathways in metabolism, such as inflammatory processes. Particularly, the non-psychotropic CBD shows great potential as an alternative treatment option. CBD is abundant in the cannabis plant, and exhibits strong antioxidatant and anti-inflammatory properties. It has been observed to cause minimal to no side effects when administered topically or systemically. Multiple studies have exhibited the anti-inflammatory properties of CBD. Mechanistically, this is achieved through its modulation of the TLR4 signal transduction cascade and changed activation of transcription factors such as NF-κB or activating protein-1. Consequently, CBD reduces the production of pro-inflammatory cytokines, including TNF-α [69]. The interaction between endocannabinoids and phytocannabinoids with cellular receptors in the metabolism has revealed the existence of a complex network known as the endocannabinoid system (ECS). This system encompasses cellular receptors, lipid signaling molecules, and metabolizing enzymes, forming an intricate framework within the body [70]. Initially, ECS was thought to be primarily present in the peripheral and central nervous systems. However, it has become evident that the ECS extends its reach to various organs throughout the metabolism. This includes not only the skin but also the gastrointestinal system and other organs. The widespread distribution of the ECS highlights its significant role in regulating numerous physiological processes beyond the nervous system. Any disruptions or imbalances in this intricately regulated system can potentially contribute to the development of pathological inflammatory conditions.

Conclusions

Hemp seed oil underwent evaluation through a series of in vitro bioevaluation tests to assess its antioxidant capabilities and its potential inhibitory effects on some metabolic enzymes. These enzymes, including AChE, α-amylase, and CA II enzymes, are linked to conditions such as AD, diabetes, and glaucoma, respectively. Furthermore, the potential active constituents within hemp seed oil were identified using LC–HR/MS, GC–FID, and GC/MS techniques. The most prevalent phenolic components discovered in the oil were apigenin, quillaic acid, and ascorbic acid. Additionally, through GC–FID analysis of hemp seed oil, it was established that 34.62 and 33.21% of the oil consisted of linoleoyl chloride and linoleic acid, respectively. These findings underscore that hemp seed oil can offer a significant and practical source of biologically significant biomolecules. Furthermore, hemp seed's oil is abundant in natural phenolic compounds, including 2,4-di-tert-butylphenol, palmitic acid, stenol, and cetal. The LC–HR/MS evaluation revealed that hemp seed oil contains substantial quantities of natural phenolic compounds,

including penduletin, luteolin-7-rutinoside, penduletin, salicylic acid, and naringenin.

Abbreviations

C. sativa	*Cannabis sativa*
GC/MS	Gas chromatography–mass spectrometry
GC–FID	Gas chromatography–flame ionization detector
LC–HRMS	Liquid chromatography–high-resolution mass spectrometry
ACh	Acetylcholine
AChI	Acetylthiocholine iodide
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
ChEIs	Cholinesterase inhibitors
DTNB	5,5′-Dithio-bis-(2-nitrobenzoic acid)
hCA II	Carbonic anhydrase II isoenzymes
$DPPH^{\cdot}$	1,1-Diphenyl-2-picrylhydrazyl radicals
$ABTS^{\cdot+}$	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radicals
TPTZ	2,4,6-Tri(2-pyridyl)-S-triazine
T2DM	Type-2 diabetes mellitus
IC_{50}	Half maximal inhibitory concentration
Fe^{2+}	Ferrous ions
Fe^{3+}	Ferric ions
Cu^{2+}	Cupric ions
ROS	Reactive oxygen species
AD	Alzheimer's disease
CO_2	Carbon dioxide
HCO_3^-	Bicarbonate ions
H^+	Protons
BHA	Butylated hydroxyanysole
BHT	Butylated hydroxytoluene
Neocuproine	2,9-Dimethyl-1,10-phenanthrolin
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
α-Tocopherol	(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1-benzopyran-6-ol
HPLC	High-performance liquid chromatography
CNS	Central nervous system
LOQ	Limit of quantification
RSC	Radical scavenging content
HCl	Hydrochloric acid
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
FRAP	Ferric ions reducing antioxidant power
CUPRAC	Cupric ions reducing antioxidant capacity
Δ_9-THC	Delta-9-tetrahydrocannabinol
CBD	Cannabidiol
ECS	Endocannabinoid system
FTIR	Fourier transform infrared spectroscopy
HPLC–MS/MS	Liquid chromatography–tandem mass spectrometry
HMBC	Heteronuclear multiple-bond correlation spectroscopy

Acknowledgements
S. Alwasel would like to extend his sincere appreciation to the Researchers Supporting Project (RSP-2024/59) of King Saud University, Saudi Arabia.

Author contributions
All authors contributed to the study's conception and design. İlhami Gulcin was responsible for supervision, conceptualization, data curation, investigation, writing—original draft, and writing—reviewing and editing. Eda Mehtap Ozden, Muzaffer Mutlu, and Zeynebe Bingol carried out investigation. Ziba Mirzaee was involved in writing—reviewing and editing, and formal analysis. Ekrem Köksal took part in conceptualization and writing—reviewing and editing. Saleh Alwasel participated in writing—reviewing and editing and visualization. Ahmet C. Goren contributed to writing—reviewing and editing, visualization, and data curation.

Funding
This research has not received external funding.

Availability of data and materials
All data generated during this study are available as a part of this article and no additional source data are required.

Declarations

Ethics approval and consent to participate
There are no studies in our article that require ethics committee approval.

Consent for publication
We certify this manuscript has not been published elsewhere and is not submitted to another journal. All authors have approved the manuscript and agreed to submit it to Future Journal of Pharmaceutical Sciences.

Competing interests
The authors declare that they have no competing interests.

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Received: 3 January 2024 Accepted: 10 September 2024
Published online: 20 September 2024

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