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Bioactive Root Extracts of *Cannabis sativa* Cultivars: Antioxidant and Anti-AChE Properties

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ABSTRACT

Cannabis sativa roots represent a historically significant yet scientifically understudied plant part, despite their traditional medicinal use and potential as a source of bioactive compounds. This study investigates the antioxidant and acetylcholinesterase (AChE) inhibitory activities of root extracts from three *C. sativa* cultivars— 'Early Remedy' (ER) is a high-CBD, low-THC medical cultivar. 'Siskiyou Gold' (SG) contains high levels of CBD and has a herbal, woody aroma, making it ideal for extraction, whereas 'Kroeng Krawia' (KK) is a tall, slender Thai landrace valued for research and economic potential. Sequential extraction was performed using solvents of increasing polarity (*n*-hexane, ethyl acetate, and methanol). Total phenolic content (TPC), total flavonoid content (TFC), and chemical profiles were assessed using standard assays and GC–MS analysis, with Trolox and galantamine serving as positive controls. The methanolic extract of KK-M showed the highest TPC (65.43 ± 0.73 mg GAE/g) and antioxidant activity (IC_{50} = 200.46 ± 6.87 µg/mL), while SG-M exhibited the strongest AChE inhibition (IC_{50} = 0.010 ± 0.002 mg/mL). GC–MS profiling of KK-M and KK-H revealed a rich array of sterols, triterpenoids, and fatty acid esters. These findings underscore the influence of solvent polarity and cultivar on extract composition and bioactivity. Importantly, they highlight the untapped therapeutic potential of *C. sativa* roots and the need to conserve phytochemical and genetic diversity for sustainable bioprospecting.

Keywords: *Cannabis sativa*, Root Extract, Antioxidant Activity, Acetylcholinesterase, Cultivars

Introduction

Cannabis sativa L. (Cannabaceae) is a widely distributed medicinal plant exhibiting notable morphological and phytochemical diversity, influenced by geography and cultivation practices. While its aerial parts—especially flowers and leaves rich in Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD)—have been extensively studied, the roots remain underexplored despite their traditional use in treating fever, pain, inflammation, and wounds.[1, 2] The plant's ecological adaptability contributes to intraspecific variation affecting the biosynthesis of secondary metabolites, with important implications for both drug discovery and biodiversity conservation.[3] Phytochemical investigations have identified triterpenoids (e.g., friedelin), sterols (e.g., β-sitosterol), and phenolic compounds in the roots, which exhibit antioxidant, anti-inflammatory, and neuroprotective activities. [4, 5, 6] Considering concerns over genetic erosion from monoculture, profiling root phytochemistry across cultivars can support sustainable resource use and expand cannabis's therapeutic 'Early Remedy' (ER), a high-CBD, low-THC medical cultivar offering therapeutic benefits without psychoactive effects; 'Siskiyou Gold' (SG), also rich in CBD with a distinctive herbal and woody aroma, suitable for extraction and incorporation into health products; and 'Kroeng Krawia' (KK), a Thai landrace variety characterized by tall, slender growth and unique local genetic traits, representing strong potential for both research and economic development. Neurodegenerative diseases, such as Alzheimer's disease, are strongly associated with oxidative stress and a reduction in the levels of the neurotransmitter acetylcholine in the brain.[8] Recent studies have highlighted the potential of natural compounds possessing both antioxidant and AChE inhibitory activities to prevent or slow neuronal degeneration by mitigating oxidative damage and enhancing cholinergic function.[9] Key bioactive constituents responsible for these effects include phenolics, flavonoids, and terpenes, which are commonly found in various medicinal plants. Among these, the roots of *C. sativa* have garnered increasing scientific interest as a source of biologically active compounds such as friedelin and epifriedelanol, which have demonstrated antioxidant and anti-inflammatory properties.[1] Consequently, the exploration of natural acetylcholinesterase (AChE) inhibitors remains a critical area of focus in neuropharmacological research.

Solvent extraction remains a fundamental and widely used technique for isolating bioactive compounds from plant materials. The type of solvent used – such as methanol, ethyl acetate, or hexane – significantly affects the yield and chemical profile of the extract due to differences in solvent polarity. Polar solvents like methanol are effective in extracting phenolic compounds, while non-polar solvents such as hexane are better suited for lipophilic substances such as hydrocarbons.[10, 11] Consequently, comparing different solvents is essential for identifying the most suitable method to maximize the recovery of biologically active constituents.

Gas chromatography–mass spectrometry (GC–MS) is a well-established analytical method that combines the separation power of gas chromatography (GC) with the structural elucidation capabilities of mass spectrometry (MS). It is extensively used to identify and quantify volatile and semi-volatile organic compounds in complex plant-based matrices.[12] The process involves vaporizing analytes, separating them

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based on their volatility and interaction with the GC column, and subsequently ionizing and fragmenting the compounds in the mass spectrometer to determine their molecular weights and structural characteristics.[13, 14] GC–MS provides detailed chemical profiles by generating retention time data and unique mass spectra, which can be matched against spectral libraries for accurate compound identification. Due to its high sensitivity, reproducibility, and broad detection range, GC–MS is considered an essential tool in phytochemical studies, particularly for detecting low-abundance secondary metabolites associated with the therapeutic efficacy of medicinal plants.[12, 15] Although our initial plan was to perform GC–MS only on the most biologically active extracts, we extended the analysis to all solvent fractions across cultivars to gain a more comprehensive understanding of their phytochemical diversity. This broader approach allowed us to identify potentially valuable compounds even in extracts that exhibited limited biological activity in preliminary assays. In recent years, the roots of *Cannabis sativa*, although historically overlooked, have gained recognition as a source of bioactive constituents such as friedelin, epifriedelanol, and other pharmacologically relevant triterpenes.[1, 16] Thus, GC–MS serves as a robust and informative platform for elucidating the full chemical landscape of *C. sativa* roots in support of future pharmacological and biodiversity-based research.

Based on this background, the present study aims to: (1) investigate the effects of different solvents (methanol, ethanol, and hexane) on the extraction of bioactive compounds from *C. sativa* roots, (2) evaluate the antioxidant and AChE inhibitory activities of the extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays and the Ellman's method[20], respectively, and (3) analyze the chemical composition of the most active extracts using GC–MS. To the best of our knowledge, this is the first comparative study focusing on the phytochemical profiling and bioactivities of *C. sativa* root extracts from selected cultivars, thereby highlighting their underexplored pharmacological value. The findings from this study may contribute to a deeper understanding of the phytochemical profile and pharmacological potential of *C. sativa* roots, ultimately supporting the development of herbal-based therapeutic products targeting oxidative stress and neurodegenerative disorders.

Materials and Methods

Chemicals and Reagents

The extraction solvents *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) were purchased from LABSOLV (Bangkok, Thailand). Folin–Ciocalteu reagent (analytical grade; Merck, Germany), sodium carbonate (Na_2CO_3, analytical grade; Merck, Germany), gallic acid (≥ 98%, analytical grade; Sigma-Aldrich, USA), aluminum chloride ($AlCl_3$) solution (Sigma-Aldrich, USA), quercetin (≥ 98%, analytical grade; Sigma-Aldrich, USA), and DPPH solution (≥ 95%, analytical grade; Sigma-Aldrich, USA) were used for antioxidant analyses. Methanol, dichloromethane (CH_2Cl_2), and water (analytical reagent grade; Fisher Scientific, UK) were used throughout the analytical procedures.

The galantamine standard was obtained from the United States Pharmacopeial Convention (USP, Switzerland). Acetylcholinesterase enzyme from *Electrophorus electricus*, Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid, DTNB), acetylthiocholine iodide (ATCI), bovine serum albumin (BSA), sodium phosphate monobasic, and disodium hydrogen phosphate dihydrate were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

Plant materials and extraction

The air-dried roots of three *C. sativa* cultivars – 'Early Remedy' (ER), 'Siskiyou Gold' (SG), and 'Kroeng Krawia' (KK) – were collected in December 2022 from cultivated populations of *C. sativa* at Tenrain Co., Ltd. (14.442936° N, 100.986002° E), located in Charoentham Subdistrict, Wihan Daeng District, Saraburi Province 18150, Thailand. Voucher specimens of *C. sativa* were prepared and deposited at the Department of Cannabis Health Sciences, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand, with the following voucher specimens: ER (SSRU-SC001), SG (SSRU-SC002), and KK (SSRU-SC003) (Figure 1).

The powdered roots – 19.3 g (ER), 150 g (SG), and 95.3 g (KK) – were sequentially extracted at room temperature using *n*-hexane (H), EtOAc (E), and MeOH (M). The solvent was subsequently filtered and evaporated to dryness to obtain the crude extract. This extraction procedure was repeated three times to ensure maximum yield. For the ER cultivar, the dried *n*-hexane (ER-H), EtOAc (ER-E), and MeOH (ER-M) extracts yielded 0.15 g, 0.64 g, and 0.87 g, respectively. For the SG cultivar, the corresponding yields were 1.76 g (SG-H), 1.09 g (SG-E), and 2.56 g (SG-M). The KK cultivar produced 1.54 g (KK-H), 1.38 g (KK-E), and 1.31 g (KK-M) of dried extracts.

Figure 1: Morphological characteristics of three *Cannabis sativa* cultivars. Stem morphology of *C. sativa* cultivars—'Early Remedy' (SSRU-SC001), 'Siskiyou Gold' (SSRU-SC002), and 'Kroeng Krawia' (SSRU-SC003), respectively; (b1–b3) Inflorescence structures of the same cultivars in the same order.

Chemical composition analysis

Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical constituents of root extracts from three *C. sativa* cultivars were characterized using a Shimadzu GC-MS-QP2020 system fitted with an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium served as the carrier gas, maintained at a constant flow rate of 1.0 mL/min. Each sample (1 µL) was introduced via split injection with a split ratio of 1:20. The oven temperature was programmed in multiple stages: starting at 70 °C with a 2-min hold, followed by a ramp of 5 °C/min to 200 °C (held for 20 min), then increased to 230 °C (held for 15 min), followed by a rise to 250 °C (held for another 15 min), and finally elevated to 320 °C, where it was held for 20 min. The ion source temperature was maintained at 250 °C under electron impact (EI) ionisation mode. Total ion chromatograms (TIC) were acquired by scanning over a mass range of 35–500 amu. Compound identification was achieved by comparing the acquired mass spectra with reference spectra in the NIST17 mass spectral library.[30]

Total phenolic content determination

The total phenolic content was determined based on the Folin–Ciocalteu method. The Folin–Ciocalteu reagent was first diluted tenfold with distilled water. Root extracts of *C. sativa* at various concentrations (5, 10, 25, 50, 100, 250, 500, 1000 µg/mL) were then mixed with the diluted Folin–Ciocalteu reagent in a 96-well microplate and incubated for 5 min. Subsequently, 7% sodium carbonate solution was added to each well, and the mixtures were incubated at room temperature for 30 min. Following the incubation period, absorbance was recorded at 760 nm using a microplate reader (SPECTROstar Nano S/N 601-1829, Ortenberg, Germany). The total phenolic content was determined and reported as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). Each analysis was conducted in triplicate, adhering to standardized protocols.[17, 18]

Total flavonoid content estimation
Total flavonoid content was assessed using the aluminium chloride colorimetric assay. *C. sativa* root extracts at varying concentrations (5, 10, 25, 50, 100, 250, 500, 1000 µg/mL) were mixed with a 2% aluminium chloride solution in a 1:1 ratio within a 96-well microplate. The mixtures were incubated at room temperature for 20 min to allow complex formation. After incubation, absorbance was recorded at 415 nm using a microplate reader. Flavonoid content was calculated and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g), all measurements were performed in triplicate.[19]

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay
The antioxidant activity was assessed using the DPPH radical scavenging assay. *C. sativa* root extracts were diluted to various concentrations (5, 10, 25, 50, 100, 250, 500, 1000 µg/mL) and mixed in equal volumes with a 0.2 mM DPPH solution in a 96-well microplate. The mixtures were then incubated in the dark at room temperature for 30 min. Following incubation, absorbance was measured at 517 nm using a microplate reader. All experiments were conducted in triplicate, with Trolox used as the positive control. Antioxidant activity was expressed as the percentage of inhibition (% inhibition), and the IC_{50} value was determined from a linear regression curve plotting sample concentration against percentage inhibition.[19]

Anticholinesterase activity testing
Acetylcholinesterase inhibitory activity was evaluated using a spectrophotometric method adapted from the 20. Ellman assay, with minor modifications.[20] The assay was conducted in 96-well microplates. Each well contained 140 µL of 10 mM sodium phosphate buffer (pH 8.0), 20 µL of AChE enzyme solution (0.2 U/mL in the same buffer), and 20 µL of the test sample dissolved in 80% methanol, resulting in a final concentration of 0.1 mg/mL, concentrations of 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625, and 0.001953125 µg/mL). The mixture was incubated at room temperature for 15 minutes to allow enzyme–inhibitor interaction.
The enzymatic reaction was initiated by adding 20 µL of a reagent mixture consisting of 5 mM DTNB and 5 mM ATCI in a 5:1 ratio, both prepared in 10 mM sodium phosphate buffer containing 0.1% BSA. The hydrolysis of ATCI was monitored by measuring the formation of the yellow 5-thio-2-nitrobenzoate anion, produced through the reaction between DTNB and the thiocholine that was released during enzymatic substrate hydrolysis. Absorbance was recorded at 405 nm after 2 minutes of incubation at room temperature.
The percentage inhibition of AChE activity was calculated by comparing the enzymatic reaction rate of each test sample to that of the negative control, which consisted of 80% methanol in sodium phosphate buffer without the test compound. Galantamine, a well-characterized AChE inhibitor, was used as the positive control to validate the assay system and benchmark the inhibitory potency of the test extracts. All assays were performed in triplicate to ensure consistency and statistical robustness.

Results and Discussion

Phytochemical composition of Cannabis sativa root extracts by GC-MS
Gas chromatography-mass spectrometry analysis revealed a diverse range of phytochemical constituents in *C. sativa* root extracts, depending on both the solvent and cultivar. The identified compounds included fatty acid esters, aldehydes, alcohols, sterols, triterpenoids, and siloxanes – many of which are known for their bioactivities (Table 1). Across all extracts, sterols such as stigmasterol, campesterol, γ-sitosterol, stigmastanol, and ergost-5-en-3-ol were prominent, and are commonly associated with anti-inflammatory, antioxidant, and cholesterol-lowering activities.[21] Triterpenoids, including friedelan-3-one, β-amyrin, and D:A-friedooleanan-3-ol, were also detected and are noted for their anti-inflammatory, anticancer, and hepatoprotective properties.[22] Fatty acid esters and derivatives, such as hexadecanoic acid methyl ester and (Z)-methyl hexadec-11-enoate, were present and known to exhibit antimicrobial and emollient effects.[23] Additionally, siloxanes such as cyclononasiloxane, octadecamethyl- and tetracosamethyl-cyclododecasiloxane were detected; while these may originate from cosmetic or plastic-derived contamination, they are occasionally found in plant waxes.
Methanolic extracts – particularly KK-M and SG-M – yielded the most chemically rich and diverse profiles, including high levels of sterols such as stigmasterol (6.01%), γ-sitosterol (9.48%), and campesterol (1.83%) as well as triterpenoids like friedelan-3-one (33.94%) and D:A-friedooleanan-3-ol (25.74%). These compounds are known to contribute to the potential anti-inflammatory and antioxidant effects of methanolic root extracts. Hexane extracts were dominated by cyclononasiloxane, octadecamethyl-, especially in ER-H (85.75%) and SG-H (31.35%), which may be endogenous or environmental contamination. Notably, ER-H and KK-H also contained high levels of friedelan-3-one (32.38% and 26.92%, respectively), indicating that hexane is efficient for extracting non-polar triterpenoids.
Ethyl acetate extracts showed moderate chemical diversity. In KK-E, notable compounds included stigmasterol (4.18%), γ-sitosterol (11.86%), and campesterol (1.62%), suggesting that the intermediate polarity of ethyl acetate enables the extraction of both polar and non-polar components. Among the cultivars, KK consistently produced the richest phytochemical profiles across all solvents, particularly in methanolic extracts, which included multiple sterols, triterpenoids, and long-chain esters such as 2-hydroxy-3-(palmitoyloxy)propyl (Z)-hexadec-9-enoate (24.82%).
'Siskiyou Gold' was notable for unique sugar alcohols and aldehydes, including L-arabinitol (34.07%), 1,4-anhydro-D-glucitol (2.91%), and hordenine (2.82%), which may contribute to neuroactive or metabolic effects. 'Early Remedy' extracts were especially rich in triterpenoids, with elevated levels of D:A-friedooleanan-3-ol (43% in ER-H) and friedelan-3-one (40.5% in ER-M). These findings align with recent reports on the phytochemical composition of *C. sativa* roots, which also identified friedelan-3-one and β-sitosterol as major constituents using GC-MS, particularly in plants cultivated in Brazil and in aeroponically grown *C. sativa* L.[16, 24]

Total Phenolic and Flavonoid Contents
The TPC and TFC of *C. sativa* root extracts varied significantly depending on the extraction solvent and cultivar (Table 2). The methanolic extract of the KK-M exhibited the highest TPC (65.43 ± 0.73 mg GAE/g extract), indicating that methanol is highly efficient in extracting phenolic constituents. In contrast, the lowest TPC was recorded in the 'Siskiyou Gold' hexane extract (SG-H, 6.24 ± 0.47 mg GAE/g extract). Regarding TFC, the KK-H extract showed the highest value (36.07 ± 0.50 mg QE/g extract), while the SG-M extract had the lowest (2.93 ± 0.13 mg QE/g extract). These findings suggest that solvent polarity significantly influences phytochemical yield, aligning with previous studies that report enhanced extraction of phenolic compounds using polar solvents.[25]
The types and quantities of compounds identified across different *Cannabis sativa* cultivars extracted with three solvents demonstrate that both genetic and environmental factors influence the biosynthesis of secondary metabolites in the plant's roots. The distinct chemical profiles observed in the KK, SG, and ER cultivars—despite being grown under similar environmental conditions—suggest that phytochemical diversity may be an inheritable trait specific to each genotype. This variability may result from intrinsic genetic factors as well as extrinsic environmental influences such as soil composition, local climate, and cultivation practices. These findings are consistent with previous studies of *C. sativa*, which reported that the chemical composition of roots can vary across cultivars or ecological contexts.[2, 3]

Antioxidant Activity
The antioxidant potential of *C. sativa* root extracts was evaluated using the DPPH radical scavenging assay. Trolox, used as a standard antioxidant, achieved nearly 100% DPPH radical scavenging at very low concentrations, confirming its high potency. Among the methanolic root extracts of *C. sativa*, KK-M exhibited the strongest antioxidant activity, reaching over 80% inhibition at approximately 400 µg/ml, which corresponds to moderate antioxidant potential. ER-M demonstrated intermediate activity, achieving nearly 70% inhibition at

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the highest tested concentration (1000 µg/ml). SG-M showed the lowest activity among the three, though its radical scavenging effect increased steadily with concentration. These findings suggest that KK-M possesses the highest antioxidant capacity among the tested extracts, likely due to its elevated total phenolic content (Figure 2).

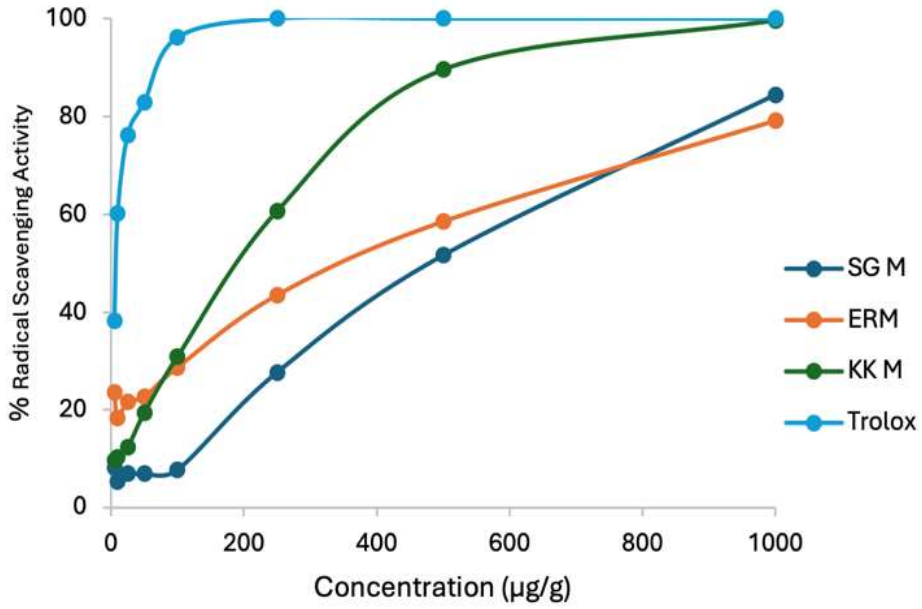

Figure 2: DPPH radical scavenging activity of extract as compared to the standard trolox.

Among all tested samples, the *n*-hexane extracts (ER-H, SG-H, KK-H) exhibited IC_{50} values exceeding 1000 µg/mL, indicating negligible radical scavenging activity. This is likely due to the low polarity of *n*-hexane, which limits its ability to solubilize polar antioxidant compounds such as phenolics and flavonoids. In contrast, the methanolic extract of KK-M exhibited the strongest antioxidant activity among all tested samples, with an IC_{50} value of 200.46 ± 6.87 µg/mL. This was followed by ER-M (IC_{50} = 454.40 ± 6.64 µg/mL) and ER-E (IC_{50} = 462.94 ± 7.64 µg/mL), both of which demonstrated moderate antioxidant activity. However, the antioxidant capacities of all extracts were markedly lower than that of the standard antioxidant compound, Trolox (IC_{50} = 5.96 ± 0.13 µg/mL), indicating the relatively limited radical-scavenging efficiency of the root-derived extracts. According to established classification, plant extracts with IC_{50} values below 100 µg/mL are considered to have strong antioxidant activity, those between 100–500 µg/mL as moderate, and values exceeding 500 µg/mL as weak or inactive.[26] The strong antioxidant performance of KK-M correlates with its high TPC, suggesting that phenolic compounds are key contributors to its radical scavenging capacity.[26, 27]

Acetylcholinesterase Inhibitory Activity

Methanolic root extracts of *C. sativa* cultivars demonstrated dose-dependent AChE inhibition (Figure 3). Galantamine, the reference inhibitor, showed >90% inhibition at low concentrations (<25 µg/mL).

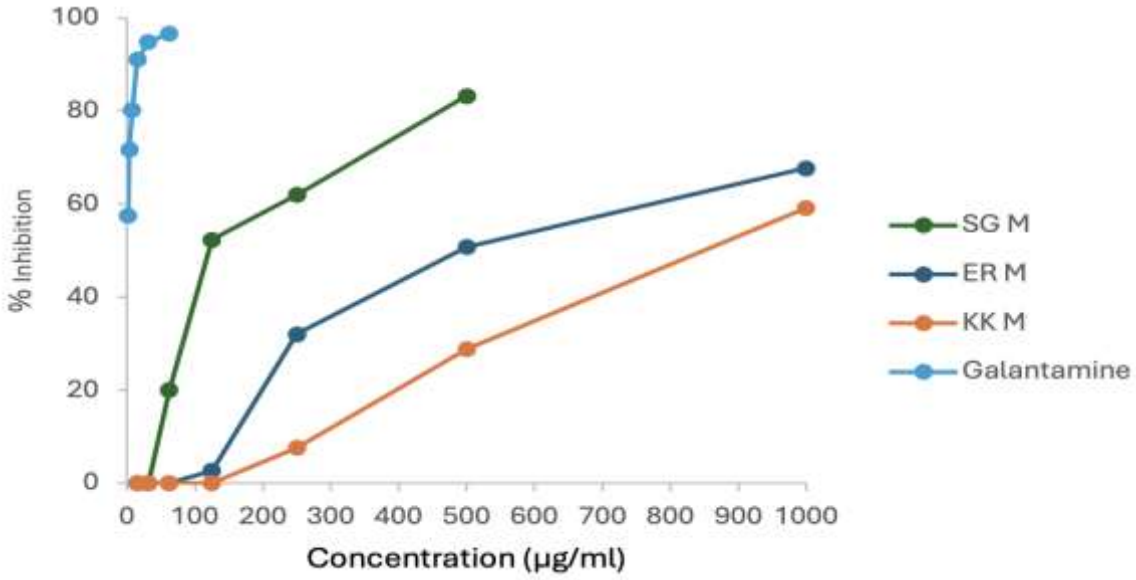

Figure 3: AChE inhibitory activity of methanolic extracts of *Cannabis sativa* roots compared to galantamine.

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Table 1: Major chemical constituents identified by GC-MS in *Cannabis sativa* root extracts from three cultivars.

Name	Retention time									Peak area%								
	ER-H	ER-E	ER-M	SG-H	SG-E	SG-M	KK-H	KK-E	KK-M	ER-H	ER-E	ER-M	SG-H	SG-E	SG-M	KK-H	KK-E	KK-M
Glycerin		5.45				5.48					8.46				7.17			
1,2,3-Propanetriol, 1-acetate		5.59				15.47					3.05				30.1			
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-		8.23		9.67	9.67		9.68	9.68			0.79		0.46	1.37		0.58	2.36	
Eicosanoic acid, 2-ethyl-2-methyl-, methyl ester		9.67									2.03							
3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-						11.10									0.4			
n-Hexadecanoic acid		17.65									2.23							
Hordenine						18.23									2.82			
D-Glucitol, 1,4-anhydro-						19.50									2.91			
Propanal, 2,3-dihydroxy-, (S)-						22.16									1.7			
L-Arabinitol						23.62									34.07			
13-Methyltetradecanal					23.90			23.91						1.57			1.04	
(Z)-Methyl hexadec-11-enoate					28.02									0.64				
Hexadecanoic acid, methyl ester					28.27									0.79				
Z,E-2,13-Octadecadien-1-ol					29.21									1.59				
Hexadecanoic acid, 2-oxo-, methyl ester					29.77									2.25				
Glycidyl palmitoleate				40.54			40.56						2.26			1.91		
Glycidyl palmitate				40.96			40.97						2.67			2.75		
Cyclohexane, eicosyl-					42.39									1.05				
1-Heneicosanol		47.14			47.12	47.13		47.15	47.15		4.15			3.49	1.29		3.94	2.04
Bis(2-ethylhexyl) phthalate		47.24									4.61							
Erucic acid						47.96									0.96			
Octacosanol		48.36			55.93			55.97			2.10			4.33			4.19	
Decanedioic acid, bis(2-ethylhexyl) ester	59.45									2.07								
Glycerol tricaprylate							65.08	65.04								9.99	4.03	

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Name	Retention time									Peak area%								
	ER-H	ER-E	ER-M	SG-H	SG-E	SG-M	KK-H	KK-E	KK-M	ER-H	ER-E	ER-M	SG-H	SG-E	SG-M	KK-H	KK-E	KK-M
Campesterol	71.2	55.95			71.14		71.16		71.15	1.75	1.38			6.46		1.62		1.83
Ergost-5-en-3-ol, (3.beta.)-				71.14		71.16		71.16					1.45		1.1		3.97	
Ergost-22-en-3-ol, (3.alpha.,5.beta.,22E)-		71.70									1.25							
Stigmasterol	71.75	71.17		71.70		71.69	71.71	71.71	71.70	6.17	3.88		1.22	5.08	1.54	2.28	4.18	6.01
22-Stigmasten-3-one	71.95				71.70					1.18								
gamma.-Sitosterol	72.87	71.92		72.82	72.82	72.80	72.83	72.83	72.80	7.01	6.73		4.41	17.18	5.84	4.44	11.86	9.48
Stigmastanol	73.05	72.82			73.01	73.00	73.01	73.03	73	2.32	2.61			2.41	1.1	0.64	2.13	2.22
3.alpha.,7.beta.-Dihydroxy-5.beta.,6.beta.-epoxycholestane		73.01									1.28							
beta.-Amyrin							73.34									1.12		
Olean-12-en-3-ol, acetate, (3.beta.)-	73.37			73.33						0.83			0.71					
4-Campestene-3-one				73.46	73.44								1.75	2.22				
Stigmasterone					73.91									1.35				
4,22-Stigmastadiene-3-one	73.94			73.91						0.85			1.47					
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)									74.39									1.40
Glutinol	74.96			74.31	74.30		74.32	74.31		1.28			1.03	0.97		2.39	1.70	
Lanosterol							75.23									0.97		
Stigmast-4-en-3-one	76.03			74.93	74.91					1.16			4.97	6.2				
D:A-Friedooleanan-3-ol, (3.alpha.)-	76.03	75.4		75.97	75.89		75.4	75.91		43.00	15.43		19.93	14.91		12.55	25.74	19.45
Cyclononasiloxane, octadecamethyl-									75.87			85.75			1.29			
Friedelan-3-one	76.30	75.9		76.30	75.39		75.93	76.2	75.38	32.38	8.67		40.47	22.79	0.96	33.94	26.92	16.68
8-Hexadecenal, 14-methyl-, (Z)-		76.17									31.35				1.1			
7-Hexadecenal, (Z)-						77.60									1.47			
Cyclononasiloxane, octadecamethyl-			76.18			78.56									1.54			
9-Octadecenal, (Z)-					79.06									1.16				
2-Undecanol oleate					79.38									2.18				

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Name	Retention time									Peak area%								
	ER-H	**ER-E**	**ER-M**	**SG-H**	**SG-E**	**SG-M**	**KK-H**	**KK-E**	**KK-M**	**ER-H**	**ER-E**	**ER-M**	**SG-H**	**SG-E**	**SG-M**	**KK-H**	**KK-E**	**KK-M**
Docosanoic acid, docosyl ester									81.24									11.42
9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester								83.48	88.02								6.25	11.39
2-Hydroxy-3-(palmitoyloxy)propyl (Z)-hexadec-9-enoate				83.49			76.27						17.2			24.82		
Hexadecanoic acid, (3-bromoprop-2-ynyl) ester									88.02									11.39
Tetracosamethyl-cyclododecasiloxane			95.12									14.25						
miscellaneous compounds														0.01	2.64		1.69	6.69

C. sativa cultivars – 'Early Remedy' (ER), 'Siskiyou Gold' (SG), and 'Kroeng Krawia' (KK) – using methanol (M), ethyl acetate (E), and *n*-hexane (H), analyzed by GC-MS, and their relative peak areas (%). In this table, ER-H, ER-E, and ER-M refer to *Cannabis sativa* root extracts from the 'Early Remedy' cultivar obtained using n-hexane, ethyl acetate, and methanol, respectively. SG-H, SG-E, and SG-M represent extracts from the 'Siskiyou Gold' cultivar, while KK-H, KK-E, and KK-M correspond to those from the 'Kroeng Krawia' cultivar, each prepared with the same solvents.

Table 2: Total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH), and anticholinesterase activity of *Cannabis sativa* root extracts

Extraction	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	DPPH IC_{50} (µg/mL)	Acetylcholinesterase IC_{50} (mg/mL) ± S.D.
ER-H	9.10 ± 0.42	32.73 ± 0.34	>1000	Inactive
ER-E	11.32 ± 0.98	36.55 ± 0.09	462.94 ± 7.64	Inactive
ER-M	20.25 ± 3.57	36.79 ± 1.38	454.40 ± 6.64	0.060 ± 0.012
SG-H	6.24 ± 0.47	37.09 ± 0.47	>1000	Inactive
SG-E	11.91 ± 0.65	20.11 ± 0.35	609.31 ± 43.09	Inactive
SG-M	36.18 ± 1.01	2.93 ± 0.13	554.82 ± 5.52	0.010 ± 0.002
KK-H	7.29 ± 0.34	36.07 ± 0.50	>1000	Inactive
KK-E	11.56 ± 0.12	33.34 ± 0.37	>1000	Inactive
KK-M	65.43 ± 0.73	18.6 ± 0.63	200.46 ± 6.87	0.085 ± 0.006
Trolox			**5.96 ± 0.13**	
Galantamine				**0.00017 ± 0.00002**

C. sativa cultivars – 'Early Remedy' (ER), 'Siskiyou Gold' (SG), 'Kroeng Krawia' (KK). Solvent extractions, Hexane (H), EtOAc (E), and MeOH (M). Different superscript letters within the same column indicate significant differences ($p < 0.05$). Acetylcholinesterase data are the average of 3 independent experiments, given as IC_{50} values in mg/mL± S.D.; Inactive at 1 mg/ml; Galantamine is the reference drug.

Among the extracts, SG-M exhibited the highest inhibitory activity, reaching over 80% at 400 µg/mL. ER-M achieved ~70% inhibition at 1000 µg/mL, while KK-M reached ~60% at the same concentration. These results indicate moderate AChE inhibitory potential, particularly for SG-M, and support the neuropharmacological relevance of *C. sativa* root constituents.

Acetylcholinesterase inhibition assays revealed that most extracts were inactive, with IC_{50} values exceeding 1 mg/mL. However, three methanolic extracts—SG-M (IC_{50} = 0.010 ± 0.002 mg/mL), ER-M (IC_{50} = 0.060 ± 0.012 mg/mL), and KK-M (IC_{50} = 0.085 ± 0.006 mg/mL)—exhibited moderate AChE inhibitory activity. Although these values were substantially weaker than that of galantamine (IC_{50} = 0.00017 ± 0.00002 mg/mL), a clinically approved AChE inhibitor, the findings highlight the potential of *C. sativa* roots as a natural source of cholinesterase inhibitors. The observed activity may be attributed to the presence of alkaloids, flavonoids, or terpenoids in the methanolic extracts, consistent with previous phytochemical studies.[28] Based on established neuropharmacological screening thresholds, inhibitory activity was classified as strong (IC_{50} < 0.1 mg/mL), moderate (0.1–1 mg/mL), or inactive (>1 mg/mL).[29]

Our results underscore the significant impact of both cultivar selection and solvent polarity on the chemical composition and biological activities of *C. sativa* root extracts. Methanol, owing to its high polarity, proved to be the most effective solvent for extracting phenolic and flavonoid compounds. Among the cultivars tested, KK exhibited the highest total phenolic content and demonstrated the most pronounced antioxidant and AChE inhibitory activities, suggesting its potential as a source of therapeutic agents for managing oxidative stress and neurodegenerative disorders. These findings are consistent with previous studies on other parts of *C. sativa*, which reported moderate antioxidant activity in ethanol root extracts—albeit lower than in leaf or flower extracts—while noting that cannabinoid-rich floral extracts exhibited greater AChE inhibitory activity than those from roots.[17, 31] Similar patterns have been observed in related species such as *Humulus lupulus*, where polar extracts from roots or stems demonstrated moderate antioxidant and neuroprotective effects.[5] These comparisons reinforce the potential of *C. sativa* roots—often underexplored—to meaningfully contribute to the plant's therapeutic profile, particularly when optimized through cultivar selection and extraction strategies. In light of their chemical richness and demonstrated bioactivity, *C. sativa* roots warrant further investigation as a phytopharmaceutical resource. Their incorporation into traditional medicine and functional nutraceuticals could expand the utility of cannabis beyond its aerial parts. Furthermore, leveraging chemotaxonomic insights alongside optimized extraction protocols may enhance the targeted development of root-based formulations with specific biological effects.[2]

Conclusion

This study provides the first comparative analysis of *C. sativa* root extracts from three cultivars 'Early Remedy' (ER), 'Siskiyou Gold' (SG), and 'Kroeng Krawia' (KK)—using solvents of different polarity. Methanolic extracts, particularly from KK, demonstrated the richest phytochemical composition and the highest phenolic content, which correlated with moderate antioxidant activity. SG extracts were notable for their comparatively stronger acetylcholinesterase inhibitory effects, while ER was especially rich in triterpenoids. These findings highlight the influence of both cultivar genetics and solvent polarity on the chemical and biological properties of *C. sativa* roots. Future prospects of this research point toward the potential of *C. sativa* roots as a valuable but underexplored phytopharmaceutical resource. Further studies should focus on the isolation and characterization of individual bioactive compounds, validation of their therapeutic efficacy through in vivo and clinical investigations, and optimization of extraction and formulation strategies. Such efforts may enable the development of root-based nutraceuticals and phytopharmaceuticals targeting oxidative stress, neurodegenerative disorders, and related health conditions. Beyond therapeutic relevance, the valorization of root-derived compounds could also expand the economic value of *C. sativa* by promoting sustainable and holistic utilization of the entire plant.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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