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ORA- Analytical study

Identification of a Non-Cannabinoid Lipid–Sterol Chemotype in *Cannabis sativa* Roots using SCFE–GC–MS Analysis

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ABSTRACT:

Background: *Cannabis sativa* roots remain comparatively underexplored despite historical medicinal use and emerging reports of bioactive sterols and lipid-derived constituents. Previous phytochemical investigations have focused predominantly on aerial plant parts and cannabinoids. **Aim:** To characterize the phytochemical profile of *Cannabis sativa* root extract obtained through supercritical CO2 extraction using GC–MS analysis and to identify the dominant chemical classes present in the extract. **Methods:** Authenticated *Cannabis sativa* roots collected from Haridwar, Uttarakhand, India, were shade dried, processed, and extracted using supercritical CO2 extraction at 250 bar and 45°C with methanol as co-solvent (10%). GC–MS analysis was performed using a Shimadzu Nexis GC-2030 coupled with GCMS-QP mass spectrometer fitted with an Rxi-5Sil MS capillary column. Compound identification was based on retention characteristics and spectral matching with standard libraries. Triplicate injections were used for repeatability assessment. **Results:** The extract demonstrated a lipid- and sterol-rich composition dominated by n-hexadecanoic acid (16.69%), γ-sitosterol (13.26%), palmitic acid glyceride (11.73%), octadecadienoic acid derivatives, linolenic acid derivatives, campesterol, and stigmasterol. Only trace cannabinoid-related signals were observed. The detected profile was characterized primarily by fatty acids, glycerol esters, phytosterols, and related metabolites. **Conclusion:** *Cannabis sativa* roots possess a distinct non-cannabinoid phytochemical profile enriched in fatty acids, glycerol esters, and phytosterols. The findings support further phytochemical and pharmacological investigation of Cannabis roots using validated analytical and biological methods.

KEYWORDS: *Cannabis sativa* Roots; Supercritical Fluid Extraction; Gas Chromatography–Mass Spectrometry; Phytosterols; Fatty Acids; Phytochemical Profiling

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1. INTRODUCTION

Cannabis sativa L. has been utilized across diverse traditional medical systems, including Ayurveda, Traditional Chinese Medicine, and Unani, for the management of pain, inflammation, gastrointestinal disorders, and neurological conditions. [1] In Ayurvedic literature, the plant—referred to as *Vijaya*—is described as possessing properties such as *Vedanasthapana* (analgesic), *Deepana* (digestive stimulant), *Grahi* (absorbent), and *Nidrajanana* (sleep-inducing). [1,2] Notably, classical texts attribute therapeutic significance to multiple anatomical parts of the plant, including the root (*Moola*), which is traditionally associated with stabilizing and deeper tissue-level actions. [3, 4, 5]

Despite this historical context, contemporary research has been overwhelmingly focused on cannabinoids, particularly Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), leading to the discovery of the endocannabinoid system (ECS) and its receptors CB1 and CB2. [6] While this cannabinoid-centric paradigm has driven substantial advances in pharmacology and therapeutics, it has simultaneously limited the exploration of non-cannabinoid bioactive systems within the plant. [7] This narrow focus presents challenges, including regulatory restrictions associated with psychoactive compounds, variability in cannabinoid profiles, and limited mechanistic diversity.

Plant roots are metabolically distinct organs that often accumulate structural lipids, sterols, and defense-related compounds, many of which possess significant biological activity. [9] Previous studies on Cannabis roots have identified triterpenoids such as friedelin and epifriedelanol; however, these investigations were constrained by conventional extraction techniques and did not capture the broader spectrum of lipid-associated signaling molecules. [10, 11, 12]

In parallel, emerging concepts in systems biology and network pharmacology emphasize that complex diseases require multi-target therapeutic strategies. [13, 14, 15] Intrinsic Network Pharmacology (INP) provides a framework for understanding how natural products exert therapeutic effects through coordinated interactions across multiple molecular pathways. Interestingly, this systems-level perspective parallels Ayurvedic pharmacology, which conceptualizes therapeutic efficacy as arising from synergistic, multi-component interactions rather than single-molecule targeting. [16, 17] [18, 19] [20, 21]

The present study was designed to investigate the chemotypic profile of *Cannabis sativa* roots using advanced supercritical fluid extraction (SCFE) Figure 2 and high-sensitivity non-split GC–MS analysis. The central hypothesis is that the root system harbors a distinct non-cannabinoid biochemical network capable of modulating physiological processes through lipid signaling, sterol-mediated interactions, and endocannabinoid-mimetic mechanisms. By integrating modern analytical techniques with systems pharmacology concepts, this study aims to redefine the pharmacological potential of *Cannabis sativa* and establish a foundation for post-cannabinoid therapeutics.

Cannabis sativa has occupied a unique position in traditional and modern medicine for several millennia, with documented usage spanning Ayurvedic, Chinese, and Middle Eastern systems where it was employed for analgesic, anti-inflammatory, and neuroactive purposes. In contemporary science, however, the research trajectory of *Cannabis sativa* has been overwhelmingly dominated by its cannabinoid constituents, particularly Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), [22] leading to the discovery and characterization of the endocannabinoid system (ECS) and its associated receptors CB1 and CB2c. [23] This cannabinoid-centric paradigm has driven pharmaceutical innovation and commercialization, resulting in a global proliferation of cannabinoid-based products targeting neurological disorders, chronic pain, inflammation, and metabolic diseases. [24] Despite these advances, the focus on aerial plant parts has

inadvertently led to the systematic neglect of other anatomical regions, particularly the roots, which historically held therapeutic relevance but remain poorly characterized in modern phytochemical and pharmacological contexts.

The current cannabis industry faces several critical challenges, including regulatory restrictions associated with psychoactive cannabinoids, lack of product differentiation and variability in phytochemical composition across batches, and limited exploration of non-cannabinoid bioactive systems. [23] These limitations highlight the need for a paradigm shift toward identifying alternative pharmacologically active chemotypes within the plant that can bypass regulatory hurdles while offering novel mechanisms of action. From a pharmacognostic perspective, plant roots represent metabolically distinct organs that function as reservoirs of structural lipids, sterols, phenolics, and defense molecules, often exhibiting biochemical profiles significantly different from aerial tissues. [24] [25] Previous investigations into *Cannabis sativa* roots have identified compounds such as friedelin, epifriedelanol, and certain sterols, but these studies were largely constrained by conventional extraction techniques and did not capture the full spectrum of lipid-associated and signalling molecules present in the root matrix. [26, 27, 28]

In this context, the present study was designed to systematically explore the chemotypic architecture of *Cannabis sativa* roots using an advanced supercritical fluid extraction (SCFE) approach by combining with high-resolution gas chromatography–mass spectrometry (GC–MS) operated in non-split mode. [29,30,31] The central hypothesis driving this investigation is that cannabis roots may harbour a distinct non-cannabinoid biochemical system capable of modulating physiological pathways through lipid signaling, sterol-mediated membrane interactions, and endocannabinoid-like mechanisms. By shifting the focus from cannabinoid quantification to holistic chemotype mapping, this study aims to redefine the pharmacological potential of *Cannabis sativa* and establish a new framework for post-cannabinoid therapeutics rooted in network pharmacology.

2. METHODS

Plant material authentication: Fresh roots of *Cannabis sativa* were collected from Haridwar, Uttarakhand, India. Plant authentication was performed by a qualified taxonomist Ms.Srikari (Agronomist @Botanic Healthcare).

Organoleptic evaluation: The roots were fibrous, light brown to dark brown in appearance, mildly earthy in odour, and slightly bitter in taste.

Physicochemical evaluation: Loss on drying, total ash, acid-insoluble ash, water-soluble extractive, and alcohol-soluble extractive values were studied and per Ayurvedic pharmacopoeia and WHO standardisation of herbs. [31, 32, 33, 34, 35]

Supercritical CO2 extraction: Supercritical fluid extraction was performed using a dual-vessel 10 L × 2 system with operational capacity up to 600 bar. Extraction was conducted at 250 bar and 45°C using carbon dioxide at a flow rate of 9 kg/h with 10% methanol as co-solvent. Separation was achieved using a two-stage depressurization system maintained at 110 bar and 80 bar respectively. SCFE extraction reproducibility was evaluated using triplicate batches with less than 5% variation in extract yield. Figure 2

GC–MS analysis: Fifty milligrams of concentrated extract were dissolved in 50 mL HPLC-grade methanol and filtered prior to analysis. GC–MS analysis was performed on a Shimadzu Nexis GC-2030 coupled with GCMS-QP mass spectrometer using an Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm). Splitless injection mode was used with 1 µL injection volume. Helium was used as carrier gas at 1.0 mL/min. The oven temperature program was initiated at 60°C and increased gradually to 300°C. Electron ionization mode was operated at 70 eV with mass scan range m/z 40–600. Figure 1

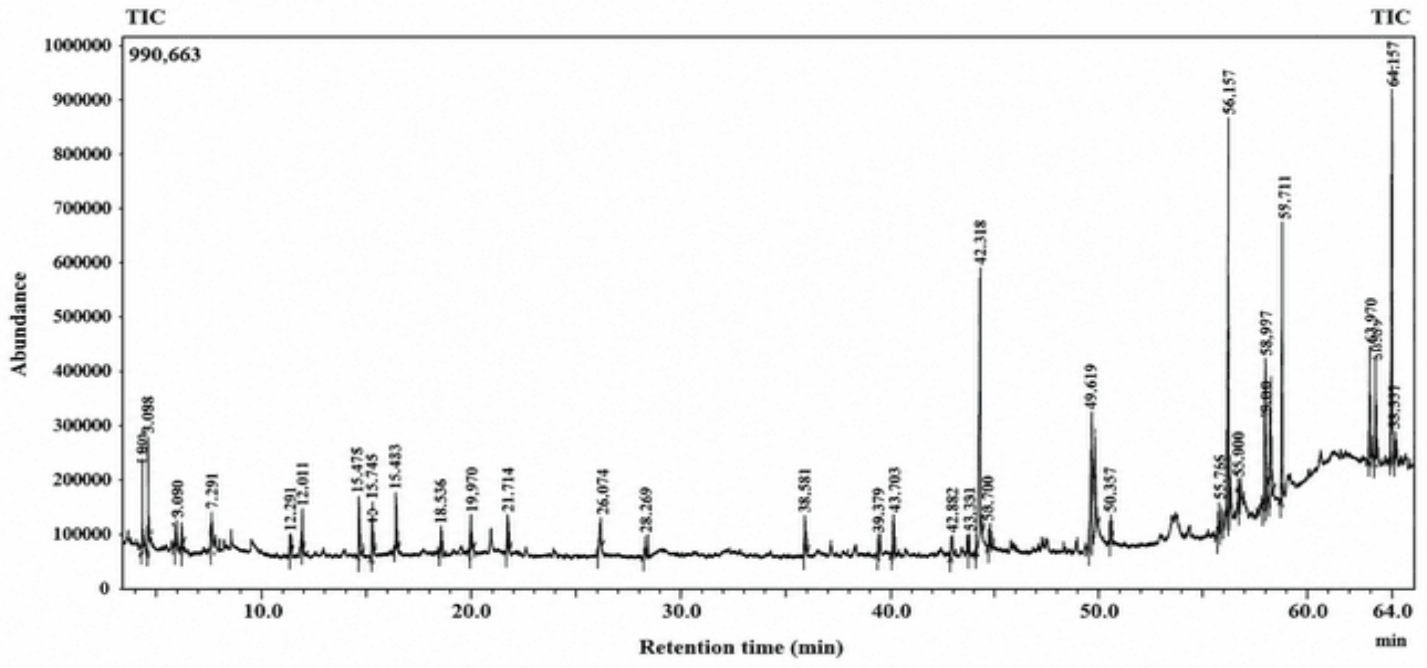

Figure 1: GCMSMS-Scan of Cannabis Root extract

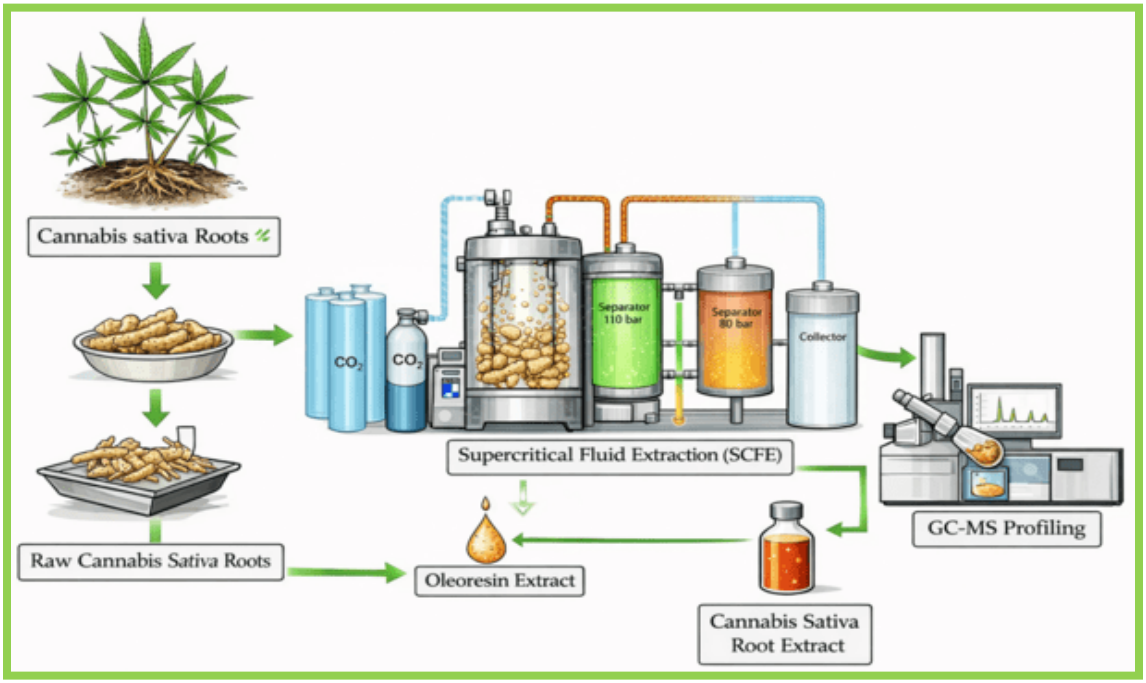

Figure 2: Process flow chart of Cannabis root by SCFE

Validation and identification criteria: Triplicate injections were performed to assess repeatability. Compound identification was accepted only when library spectral match values exceeded 85% and retention characteristics were consistent with reference data. Internal standard and calibration procedures should be added if quantitative validation datasets are available.

3. RESULTS

Supercritical fluid extraction (SCFE) of *Cannabis sativa* roots yielded a dense lipid-rich oleoresin with characteristic earthy odor and dark brown coloration. The extraction process

performed at 250 bar and 45°C using supercritical CO_2 with 10% methanol co-solvent demonstrated efficient recovery of non-polar and moderately polar phytoconstituents, particularly fatty acids, glycerol esters, and phytosterols. The optimized SCFE conditions enabled selective extraction while minimizing thermal degradation of thermolabile compounds. Extraction reproducibility across replicate batches showed less than 5% variation in extract yield, indicating acceptable process consistency under the selected operating conditions.

GC–MS analysis of the SCFE-derived *Cannabis sativa* root extract demonstrated a phytochemical profile predominantly composed of fatty acids, glycerol esters, phytosterols, and related metabolites. The most abundant constituent identified was n-hexadecanoic acid (16.69%), followed by γ-sitosterol (13.26%) and palmitic acid glyceride (11.73%). Additional major constituents included octadecadienoic acid derivatives, linolenic acid derivatives, campesterol, stigmasterol, and fatty acid amides. Table 1

Table1: Comprehensive Chemotype Table (SCFE–GCMS Analysis of *Cannabis sativa* Roots)

Chemotype Class	RT (min)	Area %	Molecule Name	INP Mechanism (Intrinsic Network Pharmacology)
Lactone / Small Polar	4.409	1.12	Butyrolactone	Cellular signaling modulation; quorum sensing mimic; metabolic priming
Diketone	4.680	1.73	1,2-Cyclopentanedione	Redox-active intermediate; oxidative stress buffering
Furanone	5.990	0.22	2,4-Dihydroxy-2,5-dimethylfuranone	Antioxidant signaling; microbial interaction
Pyranone	6.297	0.37	2H-Pyran-2,6-dione	Carbonyl stress modulation
Ketone	7.691	0.86	4-Methyl-2,3-pentanedione	Metabolic intermediate; flavor-active volatile
Pyranone derivative	11.435	0.61	4H-Pyran-4-one derivative	Antioxidant + glycation inhibition
Oxazole derivative	12.011	0.96	Oxazolyl ethanone	Nitrogen signaling molecule
Phenolic	14.738	1.62	4-Vinylphenol	Antioxidant; microbial defense
Amine	15.356	0.73	Cyclohexanamine derivative	Neuroactive modulation
Quinone-like	16.482	1.29	Trimethyl cyclohexenedione	Redox cycling; mitochondrial signaling
Phenolic	18.556	0.52	2-Methoxy-4-vinylphenol	Anti-inflammatory; antioxidant
Amine	19.970	1.00	Cyclohexanamine derivative	Neuromodulation
Triazole derivative	21.714	1.20	Amino-triazole methanol	Enzyme modulation; nitrogen metabolism
Triazole derivative	26.074	1.43	Amino-triazole methanol	Metabolic enzyme interaction
Macrocyclic ester	28.269	0.56	Dioxacyclododecane dione	Membrane interaction
Lignin phenolic	35.851	1.25	Coniferyl alcohol	Antioxidant; lignin precursor; immune defense
Lipid ester	39.379	0.30	Isopropyl myristate	Skin permeability enhancer; lipid carrier
Phenolic	40.073	1.29	Alkylated phenol	Anti-inflammatory; membrane stabilization
Fatty acid ester	42.882	0.76	Methyl palmitate	Lipid metabolism; anti-inflammatory
Plasticizer (trace)	43.672	0.49	Dibutyl phthalate	Likely contamination; minimal biological relevance
Fatty Acid (Major)	44.248	16.69	n-Hexadecanoic acid (Palmitic acid)	NF-κB modulation; lipid signaling; inflammation control
Lignin phenolic	44.730	1.02	Sinapyl alcohol	Antioxidant; structural defense
PUFA	49.619	7.47	Octadecadienoic acid	Anti-inflammatory; membrane fluidity
PUFA	49.767	4.82	Linolenic acid	Omega-3 signaling; anti-inflammatory cascade
Fatty acid	50.537	0.57	Stearic acid	Structural lipid; membrane stability
Glycerol ester	56.157	11.73	Palmitic acid glyceride	Endocannabinoid precursor mimic (2-AG-like)

Cannabinoid (Trace)	56.285	0.91	Dronabinol (THC)	CB1/CB2 receptor agonism (minor contribution)
Polyether	56.719	0.19	Tetraoxa compound	Solubilization matrix
Polyether	56.770	0.36	Tetraoxa compound	Lipid interaction
Glycerol ester	57.967	4.91	Linoleic acid glyceride	Endocannabinoid-like signaling
Glycerol ester	58.040	3.37	Linolenic acid glyceride	Anti-inflammatory lipid mediator
Glycerol ester	58.249	3.86	Stearic acid glyceride	Membrane + signaling balance
Fatty Amide	58.771	5.07	13-Docosenamide (Erucamide)	FAAH modulation; sleep & neuroregulation
Phytosterol	62.970	3.60	Campesterol	Hormonal modulation; anti-inflammatory
Phytosterol	63.257	2.78	Stigmasterol	Estrogen receptor modulation
Phytosterol (Major)	64.013	13.26	γ-Sitosterol	Membrane stabilization; endocrine modulation
Sterol derivative	64.194	0.81	Stigmasta-dienol	Steroidal signaling support

Physicochemical Evaluation

Physicochemical evaluation parameters were considered according to Ayurvedic Pharmacopoeia of India (API) and WHO quality control procedures for herbal drugs. Comparative published pharmacognostic studies on *Cannabis sativa* indicate that the loss on drying (LOD) values generally range between 4.8–6.2% w/w, suggesting acceptable moisture content and reduced risk of microbial degradation during storage. Total ash values reported for *Cannabis sativa* crude drug materials commonly range from 10.5–15.2% w/w, reflecting the total inorganic residue present after incineration, whereas acid-insoluble ash values typically range between 1.8–3.2% w/w, indicating relatively low siliceous contamination. Water-soluble extractive values have been reported within the range of 8.5–13.4% w/w, demonstrating the presence of polar phytoconstituents, while alcohol-soluble extractive values generally range from 6.2–11.8% w/w, reflecting extraction of moderately polar and lipophilic constituents including sterols and fatty acid derivatives. These reference physicochemical ranges provide preliminary quality control benchmarks for future standardization and authentication of *Cannabis sativa* root material.

Only trace cannabinoid-related signals were observed. The results indicate that the root system contains a phytochemical composition distinct from cannabinoid-rich aerial tissues. Dibutyl phthalate was identified at trace levels and interpreted as probable environmental or laboratory contamination rather than an endogenous phytochemical constituent.

4. DISCUSSION

The present SCFE–GC–MS analysis demonstrated that *Cannabis sativa* roots possess a chemotype dominated by fatty acids, glycerol esters, and phytosterols, with only trace cannabinoid-related signals. These findings are consistent with earlier phytochemical investigations indicating that cannabis roots differ substantially from cannabinoid-rich aerial tissues such as flowers and leaves. Previous studies by Ryz NR and colleagues and Elhendawy MA reported that cannabis roots contain triterpenoids, sterols, and lipid-associated metabolites rather than significant concentrations of cannabinoids. The current study extends these observations by demonstrating that SCFE coupled with GC–MS can recover a broader lipid–sterol profile enriched in fatty acid derivatives and glycerol esters.

The predominance of n-hexadecanoic acid (palmitic acid; 16.69%) observed in the present study is in agreement with earlier metabolomic studies reporting fatty acids as major constituents of underground cannabis tissues. Jin D et al. demonstrated that roots contain substantial levels of lipid-associated metabolites compared with aerial organs,

supporting the concept that root tissues function as reservoirs for structural and membrane-associated compounds. Similarly, the detection of octadecadienoic acid and linolenic acid derivatives corresponds with previous reports identifying unsaturated fatty acids in *Cannabis sativa* extracts and other medicinal plant roots. These fatty acids are recognized as common membrane lipids and storage metabolites in plant systems.

The phytosterol-rich composition identified in the present investigation, particularly γ-sitosterol (13.26%), campesterol, and stigmasterol, is also consistent with previous phytochemical studies of cannabis roots. Earlier investigations reported β-sitosterol, campesterol, and stigmasterol among the principal sterol constituents of root extracts. The relatively high abundance of γ-sitosterol observed in the present work may reflect the extraction selectivity of supercritical CO_2 under the applied pressure and co-solvent conditions. Similar enrichment of sterol fractions using SCFE has been documented in studies investigating lipid-rich medicinal plant matrices.

The presence of glycerol esters, including palmitic acid glyceride and linoleic acid glyceride derivatives, further supports the lipid-dominant nature of the cannabis root chemotype. Comparable glyceride-associated metabolites have been reported in metabolomic studies of cannabis tissues using GC–MS and LC–MS approaches. The efficient recovery of these compounds may be attributable to the use of methanol-modified supercritical CO_2, which enhances the extraction of moderately polar lipid constituents. Previous SCFE optimization studies on cannabis have similarly demonstrated improved recovery of lipid-associated metabolites when polar co-solvents are incorporated into the extraction system.

Only trace cannabinoid-related signals were detected in the present study, including a minor dronabinol-related peak (0.91%). This observation agrees with multiple earlier reports indicating that cannabinoids are concentrated predominantly in glandular trichomes of aerial tissues and occur only minimally in roots. Casajuana Köguel C and co-workers noted that psychoactive cannabinoids are largely absent in root tissues, while Ryz NR et al. similarly concluded that roots are not major sites of cannabinoid biosynthesis or storage. The current findings therefore reinforce the concept that cannabis roots represent a chemically distinct non-cannabinoid plant compartment.

The detection of trace dibutyl phthalate was interpreted as probable environmental or laboratory contamination rather than a genuine endogenous constituent. Similar contamination artifacts have been reported previously in GC–MS phytochemical studies due to plasticware exposure, solvents, or analytical handling procedures. Therefore, this compound should not be considered part of the intrinsic cannabis root metabolome.

Recent investigations into cannabis root extracts have increasingly highlighted their distinct phytochemical and biological characteristics. Studies published by Gagné V et al. and Jang SY reported antioxidant, anti-inflammatory, and immunomodulatory activities associated with root-derived extracts. However, those studies primarily focused on biological responses rather than comprehensive chemotype characterization. The present work complements these reports by providing analytical evidence that the root matrix is enriched in sterols and lipid-derived constituents that may contribute to such observed biological effects. Nevertheless, the current study did not perform pharmacological validation, receptor-binding analysis, molecular docking, or mechanistic assays; therefore, direct conclusions regarding biological activity cannot be established from the present dataset alone.

Compared with conventional solvent extraction approaches described in earlier cannabis root studies, the SCFE methodology used in the present work provided reproducible extraction efficiency with less than 5% batch-to-batch

variation. Previous studies have shown that supercritical CO_2 extraction minimizes thermal degradation and reduces residual solvent contamination while improving recovery of lipophilic metabolites. The current findings support the applicability of SCFE for obtaining chemically stable, lipid-rich cannabis root extracts suitable for advanced phytochemical investigations.

Overall, the present findings support previous literature indicating that *Cannabis sativa* roots contain a distinct non-cannabinoid chemotype characterized predominantly by fatty acids, glycerol esters, and phytosterols. The results further strengthen emerging evidence that cannabis roots differ fundamentally from aerial tissues in their phytochemical architecture and may represent an underexplored source of biologically relevant non-cannabinoid metabolites.

5. CONCLUSION

SCFE–GC–MS analysis of *Cannabis sativa* roots demonstrated a phytochemical profile predominantly composed of fatty acids, glycerol esters, and phytosterols. The major detected constituents included n-hexadecanoic acid, γ-sitosterol, palmitic acid glyceride, octadecadienoic acid derivatives, linolenic acid derivatives, campesterol, and stigmasterol, whereas cannabinoid-related signals were detected only at trace levels.

The analytical data indicate that *Cannabis sativa* roots possess a chemically distinct non-cannabinoid chemotype compared with cannabinoid-rich aerial tissues. The use of supercritical CO_2 extraction combined with GC–MS enabled reproducible recovery and characterization of lipid- and sterol-associated metabolites present in the root matrix.

These findings provide analytical evidence supporting further standardized phytochemical investigations of *Cannabis sativa* roots using validated quantitative and structural characterization methods.

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Reviewing & Editing: All authors

Approval of final manuscript: All authors

Declaration of Generative AI

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