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Comprehensive Phytochemical Profiling of Native *Cannabis sativa* L. from Dehloran Using HS-SPME, GC–MS, and FT-IR Techniques

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A B S T R A C T

Objective: *Cannabis sativa*, a member of the Cannabaceae family, has a long history of use in Iranian traditional medicine, particularly for pain relief, alleviating menstrual discomfort, and improving mood disorders. This study aimed to extract and characterize the phytochemical constituents of native *C. sativa* from Dehloran (Ilam Province) using headspace solid-phase microextraction (HS-SPME), gas chromatography-mass spectrometry (GC-MS), and Fourier-transform infrared (FT-IR) analytical techniques.

Methods: Plant specimens were collected from Dehloran, located in southern Ilam Province. The aerial parts of the plants were shade-dried and finely powdered. Essential oils were extracted using HS-SPME, and their chemical constituents were analyzed using GC-MS. FT-IR spectroscopy was employed to identify key functional groups. The essential oil extracted from Dehloran *C. sativa* seeds using HS-SPME was successfully analyzed with GC-MS.

Results: A total of 36 volatile compounds were identified, with β-pinene, trans-caryophyllene, and α-humulene as the primary constituents. The chemical profile included monoterpenes, sesquiterpenes, oxygenated and esterified terpenoids, as well as several non-terpenoid compounds. FT-IR analysis further validated the presence of key functional groups, such as alcohols, esters, carbonyls, and alkanes. These findings collectively emphasize the plant's richness in bioactive and antioxidant compounds, highlighting its potential as a valuable resource for pharmaceutical applications.

Conclusion: The essential oil of native *C. sativa* is rich in bioactive and antioxidant constituents, making it a promising candidate for developing medicinal formulations. The findings of this study provide a foundation for identifying pharmacologically relevant compounds in indigenous Iranian cannabis ecotypes and may aid in the advancement of natural therapeutic products.

Keywords: Cannabis sativa, Fourier Transform Infrared Spectroscopy, Gas Chromatography–Mass Spectrometry, Headspace Solid-Phase Microextraction, Dehloran ecotype, Iran

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Introduction

Medicinal plants have been vital natural resources for the prevention and treatment of various ailments since ancient times and continue to play a significant role in primary healthcare systems around the world [1, 2]. Factors such as population growth, the emergence of adverse effects related to synthetic drugs, the spread of drug resistance, and limited access to conventional chemical therapies have heightened interest in medicinal plants as affordable, safer, and more accessible therapeutic alternatives [3]. According to the World Health Organization, about 80% of the global population relies on plant-based products to address at least some of their primary healthcare needs [4].

The chemical components of medicinal plants are generally categorized into primary and secondary metabolites. Primary metabolites, including carbohydrates, lipids, and amino acids, are essential for the plant's survival. In contrast, secondary metabolites, such as alkaloids, flavonoids, glycosides, mucilage, and tannins, play crucial roles in plant defense mechanisms and exhibit a variety of biological activities [5, 6].

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In Dehloran County, located in Ilam Province, *Cannabis sativa* L., commonly known as Dehloran hemp, occupies a significant role within the local flora. This annual, dioecious plant typically grows to heights of 1 to 3 meters. Notably, its palmate leaves and oil-rich seeds have traditionally been utilized to support general health, enhance digestive function, relieve constipation and nausea, alleviate menstrual discomfort, reduce inflammation, and promote skin and hair health. Hemp seeds are especially valued for their complete protein content, essential omega-3 and omega-6 fatty acids, vitamins, and minerals. Additionally, various parts of the plant have diverse industrial applications [7-11]. Research has shown that certain compounds found in *C. sativa* may enhance immune function, modulate hormonal activity, and possess anticancer properties [12-14].

The identification and extraction of bioactive compounds from medicinal plants, particularly essential oils, play a crucial role in the development of pharmaceutical, nutraceutical, and cosmetic products. Essential oils are complex mixtures of volatile compounds, which include monoterpenes, sesquiterpenes, and oxygenated derivatives such as alcohols, aldehydes, ketones, and esters [10, 11]. Modern analytical techniques, including headspace solid-phase microextraction (HS-SPME), gas chromatography-mass spectrometry (GC-MS), and Fourier-transform infrared spectroscopy (FT-IR), enable precise characterization of the volatile constituents and functional groups within essential oils [7-9].

Medicinal plants are excellent sources of natural antioxidants, which play a crucial role in neutralizing free radicals and preventing cellular damage as well as chronic diseases [15-21]. Therefore, conducting phytochemical investigations on valuable indigenous species, such as Dehloran hemp, offers opportunities to identify bioactive molecules and promote the development of new therapeutic and dietary products.

This study focuses on identifying and characterizing the volatile compounds and functional groups in the essential oil of native Dehloran *C. sativa* using techniques such as Headspace Solid-Phase Microextraction (HS-SPME), Gas Chromatography-Mass Spectrometry (GC-MS), and Fourier Transform Infrared Spectroscopy (FT-IR).

Materials and Methods

Sampling and Identification:

Specimens of *Cannabis sativa* L. were collected from wheat fields in Dehloran County, Ilam Province, western Iran. Approximately 1 kg of fresh plant material was harvested and transported to the laboratory, where it was dried in a shaded, well-ventilated area, protected from direct sunlight. Between 100 and 200 g of the dried material was selected for chemical analysis and finely ground. Plant identification was performed based on morphological characteristics, using the *Flora Atlas of Medicinal Plants of Ilam Province*, and was further verified by the Biotechnology and Medicinal Plants Research Center at Ilam University of Medical Sciences.

Sample Preparation

The dried plant material was manually cleaned and ground into a homogeneous powder using a mixer mill. The resulting powders were stored in airtight containers under moisture-free conditions and were used for HS-SPME, GC-MS, and FT-IR analyses.

HS-SPME Extraction Procedure

Volatile compounds from the powdered hemp were extracted using HS-SPME. For each extraction, 0.5 g of powdered sample was placed in a glass vial, and 50 µL of water was added to adjust moisture content. The samples were then sonicated for 15 minutes using a Euronda ultrasonic device (Italy). Extraction was carried out at 60°C for 20 minutes. A 100 µm polydimethylsiloxane (PDMS) fiber (SUPELCO) was used, and the adsorbed compounds were desorbed into the GC-MS inlet for three minutes.

GC-MS Analysis

Volatile compounds were analyzed using an Agilent 6890N gas chromatograph coupled with an Agilent 5973 mass spectrometer. A 30 m HP-5 capillary column with an internal diameter of 0.25 mm and a stationary phase thickness of 0.25 µm, was utilized. The sample was injected in split/splitless mode. The oven temperature program consisted of an initial hold at 50°C, followed by a ramp of 5°C/min up to 180°C, and then an increase of 12°C/min up to 250°C. Helium (99.999% purity) was used as the carrier gas at a flow rate of 0.9 mL/min, and the injector temperature was set to 250°C.

Data Analysis and Kovats Index Calculation

Qualitative identification of compounds was performed by comparing mass spectra with the Wiley 7n reference library. The relative percentage of each compound was calculated based on peak areas. The Kovats retention index was determined using third-degree polynomial equations based on retention times to enhance accuracy. Data processing was conducted using Statgraph software, and major compounds were reported according to their Kovats indices and relative abundances.

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FT-IR Spectroscopy

Fourier-transform infrared (FT-IR) spectroscopy was employed to determine functional groups and investigate molecular structures in the hemp samples. Powdered samples were placed directly onto the sample holder without any pretreatment, and spectra were recorded over the range 4000–400 cm^{-1}. Absorption peaks were compared with reference data and standard libraries to identify principal functional groups, including alkanes, alcohols, carbonyls, phenols, and other relevant molecular structures.

Results

Cannabis sativa is a naturally occurring plant known for its distinctive palmate leaves. The seeds of the hemp plant have various therapeutic properties, including calming effects, enhanced immune function, and hormonal regulation. Different parts of the plant can be used to produce to produce hemp fibers, seeds, marijuana, and hashish. The small, uniform hemp seeds, with their soft texture and nutty flavor, are particularly valued. In this study, we investigated the phytochemical composition of hemp seeds using FT-IR, HS-SPME, and GC-MS techniques. This analysis (Table 1) identified a total of 36 chemical constituents in the essential oil. The major components included β-pinene (31.26%), trans-caryophyllene (18.29%), α-humulene (7.92%), dl-limonene (6.16%), linalool (5.54%), E-caryophyllene (4.80%), menthyl acetate (3.20%), and β-myrcene (3.02%). Together, these eight compounds accounted for over 80% of the total essential oil, highlighting their potential biological significance and pharmaceutical relevance.

The molecular and chemical structures of the eight main constituents are presented in Table 2, while the relative percentages of the remaining compounds are summarized in Table 3.

Table 1: Chemical Constituents of Cannabis sativa Seed Essential Oil Identified by GC-MS

No.	Retention Time (min)	Compound	Area	KI	%
1	6.21	α-Pinene	33,600,165	930	1.90
2	7.24	β-Pinene	552,860,517	981	31.26
3	7.56	β-Myrcene	53,357,661	992	3.02
4	8.08	Δ3-Carene	5,698,558	995	0.32
5	8.66	dl-Limonene	109,003,397	1028	6.16
6	9.09	Trans-Ocimene	13,906,170	1032	0.79
7	9.41	γ-Terpinene	20,531,434	1045	1.16
8	9.79	Limonene	3,950,518	1061	0.22
9	10.35	Ethyldimethylthiophene	7,566,801	1073	0.43
10	11.75	cis-Pinocarveol	8,626,857	1085	0.49
11	12.13	Linalool	98,044,121	1098	5.54
12	13.07	Acetophenon ar-methyl	19,422,849	1161	1.10

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13	13.28	Myrtenal	17,324,598	1189	0.98
14	13.87	Fenchyl acetate	10,172,721	1238	0.58
15	14.04	Carveol	3,631,495	1241	0.21
16	14.50	Pulegone	3,210,216	1247	0.18
17	14.66	Carvone	8,166,038	1251	0.46
18	14.95	Pipertone	3,543,323	1262	0.20
19	15.38	Camphane	4,459,542	1271	0.25
20	15.68	Thymol	16,166,447	1288	0.91
21	15.92	Menthyl acetate	56,650,904	1312	3.20
22	18.11	E-Caryophyllene	84,923,913	1396	4.80
23	19.30	Trans-Caryophyllene	323,452,043	1418	18.29
24	19.48	Calarene	10,540,496	1446	0.60
25	20.13	α-Humulene	139,989,556	1459	7.92
26	20.66	α-Amorphene	11,274,066	1475	0.64
27	20.83	Germacrene D	15,600,420	1485	0.88
28	20.90	β-Ionone	10,640,312	1489	0.60
29	21.23	α-Gurjunene	10,040,519	1493	0.57
30	21.40	β-Bisabolene	4,231,971	1501	0.24
31	21.63	Gamma-Cadinene	14,834,951	1523	0.84
32	21.80	Delta-Cadinene	21,429,062	1538	1.21
33	23.33	Caryophyllene oxide	34,551,511	1589	1.95
34	25.84	2,4-Dimethylpyrroline	19,645,332	1613	1.11
35	31.32	n-Hexadecanoic acid	9,773,742	1851	0.55
36	32.54	3-Octyl phenol	7,751,711	1879	0.44

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Based on the obtained results, the chemical constituents of hemp seed essential oil have been classified according to their chemical types as follows:

Monoterpenes: α-Pinene, β-Pinene, β-Myrcene, Δ³-Carene, dl-Limonene, trans-Ocimene, γ-Terpinene, and Limonene.

Sesquiterpenes: α-Humulene, trans-Caryophyllene, E-Caryophyllene, Germacrene D, and β-Bisabolene

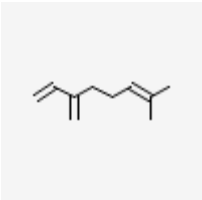
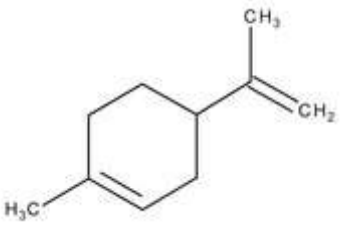
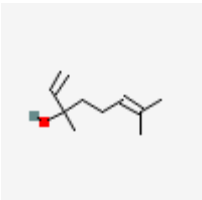
Oxygenated Terpenes: Caryophyllene oxide, Carveol, Fenchyl acetate, and Pulegone

Esterified Terpenes: Menthyl acetate.

Non-Terpenoid Compounds: n-Hexadecanoic acid, 3-Octyl phenol, and 2,4-Dimethylpyrroline

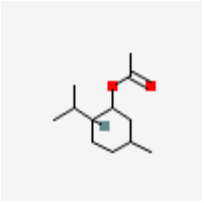
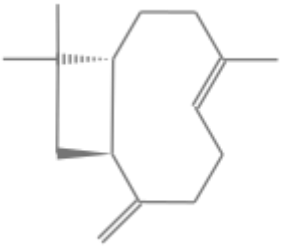
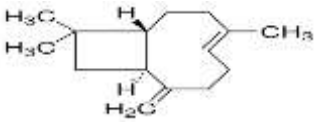
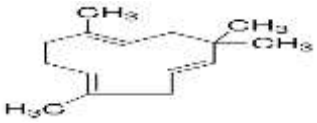
This classification provides a clearer understanding of the various chemical components present in hemp seed essential oil.

Table 2: Molecular and Chemical Structures of the Eight Major Compounds in Cannabis sativa Seed Essential Oil

Compound	Chemical formula	Chemical structure
beta.-Pinene	C10H16	
.beta.-Myrcene	C10H16	
dl-Limonene	C10H16	
linalool	C10H18O	

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menthyl acetate	C12H22O	
E- Caryophyllene	C15H24	
Trans-Caryophyllene	C15H24	
Alpha- Humulene	C15H24	

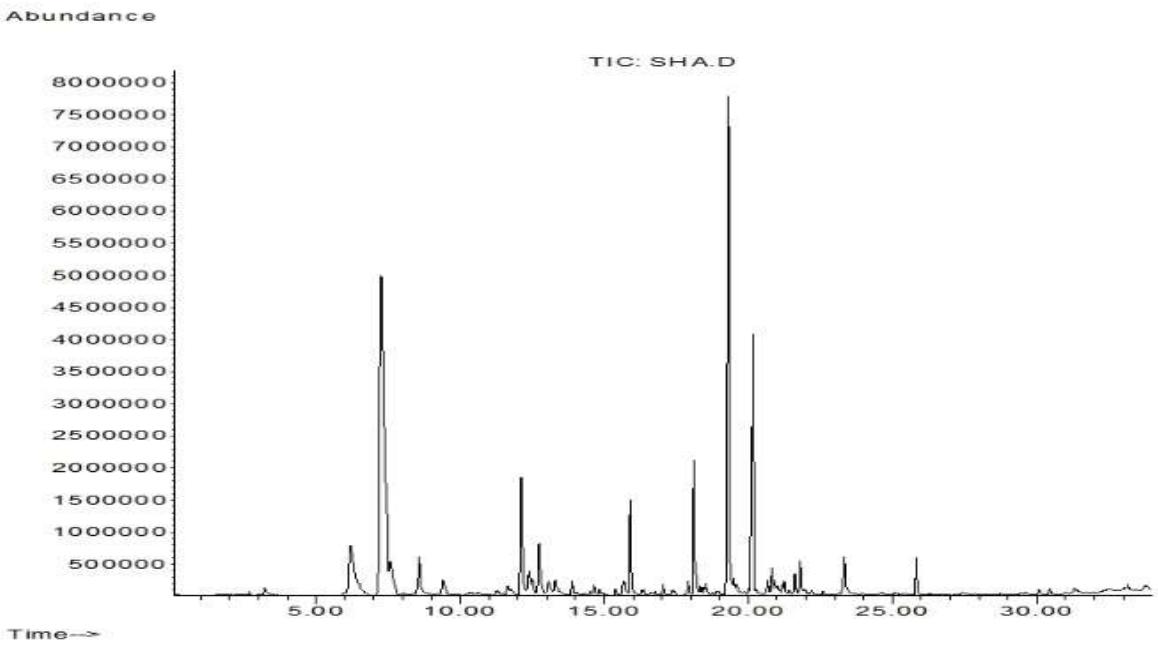


Figure 1: GC-MS Chromatogram of Cannabis sativa Seed Essential Oil

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This study focused on the identification of native Dehloran Cannabis sativa (canabis), which involved collecting, drying, powdering, and extracting its chemical constituents. The characterization of these constituents was conducted using Fourier-transform infrared (FT-IR) spectroscopy. FT-IR is a powerful analytical technique that provides the infrared absorption spectrum of a substance based on its wavenumber, thus offering insights into molecular vibrations and functional groups. Most materials absorb light in the infrared range, and analyzing their absorption spectra enables the identification of molecular components and structural features.

In this study, FT-IR spectroscopy was performed on hemp seeds. The results are illustrated in Figure 2 and Table 3. As depicted in the spectrum (Figure 2), the hemp seeds displayed 12 the distinct absorption peaks, which correspond to key functional groups present in the sample.

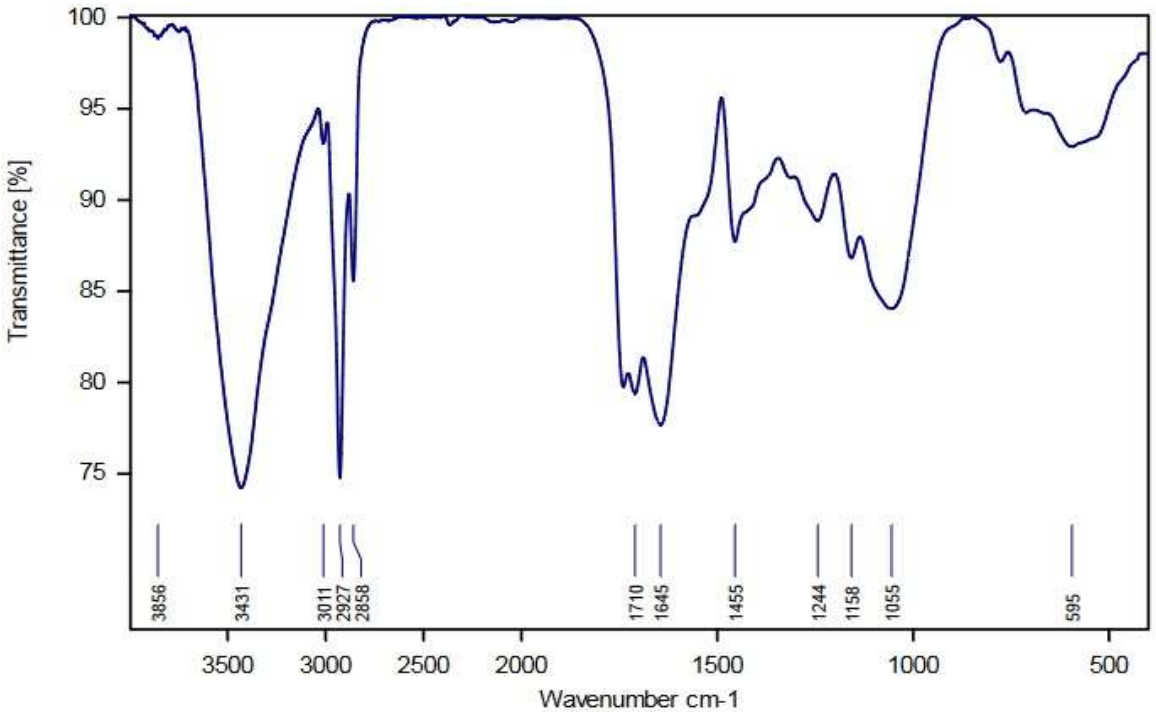


Figure 2: Fourier-Transform Infrared (FT-IR) Spectrum of Cannabis sativa Seeds

As shown in Table 3, the plant exhibited 12 distinct absorption peaks corresponding to various functional groups. FT-IR analysis revealed the presence of several chemical compounds, including alcohols, primary amines, carboxylic acids, amine salts, conjugated acids, imines/oximes, alkanes, aromatic esters, secondary alcohols, sulfoxides, and halogenated compounds. This highlights the diverse chemical composition of Cannabis sativa.

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Table 3: FT-IR Absorption Bands, Wavenumbers, Functional Groups, and Corresponding Organic Compounds in Cannabis sativa Seeds

Wavenumber (cm^{-1})	Functional Group	Compound Type	Associated Compounds
3856	C−H	Alcohol (Stretching)	Carveol, Fenchyl acetate
3431	N−H	Primary Amine (Stretching)	—
3011	O−H	Carboxylic Acid (Stretching)	—
2927–2858	N−H	Amine Salt (Stretching)	—
1710	C=O	Conjugated Acid (Stretching)	Fenchyl acetate, Pulegone
1645	C=N	Imine/Oxime (Stretching)	—
1455	C−H	Alkane (Bending)	α-Humulene, β-Bisabolene
1375	C−H	Methyl Group (Bending)	α-Humulene, β-Bisabolene
1244	C−O	Aromatic Ester (Stretching)	Menthyl acetate
1158	C−O	Secondary Alcohol (Stretching)	Carveol
1080	C−O	Ether (Stretching)	—
1055	S=O	Sulfoxide (Stretching)	—
960	C=C	Alkene (Out-of-Plane Bending)	α-Humulene
875	=C−H	Alkene (Bending)	β-Bisabolene
595	C−I	Halogenated Compound (Stretching)	—

Based on the results presented in Table 3 and the identified functional groups, the hemp seed essential oil can be broadly categorized as follows:

Oxygenated Terpenes (Caryophyllene oxide, Carveol, Fenchyl acetate, Pulegone):
FT-IR spectra indicated the presence of alcohol (C−H stretching), carbonyl (C=O), and C−O functional groups, consistent with the structures of oxygenated terpenes. For example, Carveol and Fenchyl acetate contain both alcohol and ester/carbonyl groups, which were observed within the wavelength range of 3856–1710 cm^{-1}.

Esterified Terpenes (Menthyl acetate):

The presence of the C−O ester functional group was detected at 1244 cm^{-1}, which corresponds with the chemical structure of Menthyl acetate.

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Non-Terpenoid Compounds (n-Hexadecanoic acid, 3-Octyl phenol)

The FT-IR spectra revealed the presence of carboxylic acid groups, indicated by O−H stretching at approximately 3011 cm^{-1}, as well as alkanes, shown by C−H bending at around 1455 cm^{-1}. These findings align with the identified non-terpenoid compounds.

Overall, the functional groups detected by FT-IR, including alcohols, esters, carbonyls, and alkanes, correlate well with the compounds found in hemp seed essential oil. These results confirm the chemical structures of the constituents and provide a foundation for further investigation into their bioactive properties.

Discussion

Cannabis sativa, a member of the Cannabinaceae family, is one of the most widely studied medicinal plants due to its diverse range of bioactive compounds. The majority of its active constituents are found in the resinous portions of the plant, which include cannabinoids, terpenes, alkaloids, and fatty acids. Historically, Cannabis sativa has been used to treat various conditions, such as, skin disorders, neurodegenerative diseases, headache disorders, rheumatoid arthritis. This highlights its long-standing role in traditional medicine and its multifunctional therapeutic potential [22-25].

The primary bioactive components of *C. sativa* are cannabinoids, with the most notable being tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol, cannabigerol (CBG), and cannabichromene. Additionally, hemp seed volatile oils contain a mixture of terpenes and sesquiterpenes, including cannabinenes, as well as specific alkaloids such as trigonelline [26, 27]. This chemical diversity contributes to the plant's varied therapeutic effects. In this study, the essential oil extracted from native Dehloran hemp seeds was primarily composed of several compounds, including β-Pinene, trans-Caryophyllene, α-Humulene, Limonene, Linalool, E-Caryophyllene, Menthyl acetate, and β-Myrcene. The variations in the relative abundance of these compounds may be due to factors such as genetic differences, climatic conditions, soil characteristics, harvest timing, and extraction methods. These findings highlight the significance of ecological and comparative studies in understanding the medicinal potential of this native species. Tetrahydrocannabinol (THC), the main psychoactive component of the plant, is unevenly distributed throughout the plant. The highest concentrations are found in the flowers, while leaves, stems, and seeds contain only trace amounts. Therefore, hemp seeds, which have minimal THC content, serve as a safe dietary source and are suitable for plant oil extraction. Nutritionally, hemp seeds are rich in protein (20–25%), carbohydrates (20–30%), oil (25–35%), and insoluble fiber (10–15%). They also provide essential minerals such as phosphorus, potassium, magnesium, calcium, iron, and zinc, and vitamins A, C, and E [28]. The high levels of unsaturated fatty acids, including linoleic and linolenic acids, contribute to beneficial effects on cardiovascular health, lipid regulation, and blood pressure control [29]. Additionally, certain terpenes like Myrcene and Caryophyllene possess antimicrobial properties, while cannabichromene shows antifungal and antibiotic effects [30, 31].

Hemp roots contain bioactive pentacyclic terpenes, such as friedelin and epifriedelanol, as well as amide derivatives, including N-(p-hydroxy-β-phenylethyl)-p-hydroxy-trans-cinnamamide, which add to their therapeutic potential [32,33].To date, , more than 560 chemical compounds have been identified in *C. sativa*, including more than 100 cannabinoids and a diverse array of nitrogenous compounds, amino acids, carbohydrates, hydrocarbons, terpenes, and fatty acids [34-39]. Non-psychoactive cannabinoids, such as CBD and CBG, play significant roles in managing pain and inflammation, alleviating nausea, stimulating appetite, reducing muscle spasms, and treating various conditions like epilepsy, multiple sclerosis, spinal cord injury, glaucoma, and cancer [40]. These properties make *C. sativa* as a promising candidate for novel therapeutic development and a viable alternative to conventional drugs that often have high side-effect profiles. The synergistic interactions between cannabinoids and terpenes known as the "entourage effect," further enhance the plant's therapeutic efficacy. Coupled with the high nutritional value of hemp seeds and seed oil, this makes *C. sativa* attractive appealing for functional food applications. The variability in the composition and concentration of bioactive compounds across different parts of the plant underscores the need for standardized extraction methods and region-specific research. Emerging evidence regarding the neuroprotective, anti-inflammatory, and antimicrobial effects of newly identified compounds further supports the potential of hemp in developing innovative therapies for neurodegenerative and chronic diseases.

In summary, Cannabis sativa is a versatile plant with a diverse chemical profile and high nutritional value, making it applicable in modern medicine, pharmaceuticals, and the food industry. Future research should prioritize optimizing extraction techniques, clarifying precise mechanisms of action, and conducting thorough safety assessments to support the scientifically sound and safe use of this species.

Conclusion

Cannabis sativa is a valuable resource for health and nutrition due to its rich variety of bioactive compounds, including cannabinoids, terpenes, flavonoids, fatty acids, and alkaloids.

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These constituents offer multiple therapeutic properties, such as anti-inflammatory, analgesic, antimicrobial, antioxidant, and immunomodulatory effects. Hemp seeds and their oil provide high-quality proteins and unsaturated fatty acids, highlighting their significant potential for functional food and pharmaceutical applications. The native Dehloran hemp, known for its unique terpene profile and impressive antioxidant properties, stands out as a promising source for developing herbal medicines and functional food products. To ensure safe and effective use, further studies are necessary to standardize extraction methods, clarify mechanisms of action, and conduct comprehensive safety evaluations. Such research will lay the groundwork for creating hemp-based therapeutic and nutritional products.

Declaration

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Competing interest

Not applicable.

Ethics approval

Not applicable.

Availability of data and material

Not applicable.

Code availability.

Not applicable.

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