

Chemical composition and antioxidant activity of essential oil of *Cannabis sativa* subsp. *sativa* L. from the Al-Hoceima province in northern Morocco

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Abstract. *Cannabis sativa* subsp. *sativa* L. is an industrial plant that has gained importance due to its essential oil (EO), which has commercial, medical, and potential cosmetic applications. Despite its significance, the chemical constituents of the *C. sativa* EO cultivated in Morocco is not well understood. In this context, the current study aimed to define and analyze the volatile fraction and evaluate the antioxidant capacity of the plant *C. sativa* EO for the first time in the Al-Hoceima province in northern Morocco. To assess its chemical composition, gas chromatography-mass spectrometry (GC-MS) was utilized after hydrodistillation using a Clevenger-style apparatus. The analysis revealed that caryophyllene, α -humulene, and β -myrcene were the major constituents, accounting for 31.77%, 11.21%, and 8.76% of the EO, respectively. Antioxidant activity results indicate that the potency of *C. sativa* EO is interesting ($IC_{50} = 4.45$ mg/mL) for the DPPH• (2,2-diphenyl-1-picrylhydrazyl) test. These findings indicate that the *C. sativa* EO may serve as a natural source of antioxidants. **Keywords:** chemical analyses, *C. sativa*, Mass Spectrometry, Essential oil, Antioxidant activity

1 Introduction

Cannabis, belonging to the *Cannabaceae* family, comprises three identified species (*C. indica*, *C. ruderalis* and *C. sativa*) and More than 700 varieties [1]. In Morocco, it is locally known as "El kif" and it is a seasonal angiosperm. widely grown in the north (Rif region) in temperate climates [2]. *Cannabis* has been an integral part of Moroccan culture and cultivation for centuries, with its introduction dating back to the Arab conquests of North Africa. The Rif mountains in northern Morocco became the primary growing region for *Cannabis* in the 18th century. Due to its unique location, Morocco boasts a diverse ecosystem with a vast array of plant species, including nearly 3913 species belonging to 981 genera and 155 families, forming a genuine botanical reserve [3]. The female leaves and flowers of *Cannabis* are highly valued for their secondary metabolites, including cannabinoids, flavonoids, terpenoids, alkaloids, lignans, anthocyanins, and quinones [4]. The essential oils, also called volatile fractions, are of particular interest to various industries due to their biological properties, such as antimicrobial and antioxidant effects [5]. Literature reviews have identified More than 100 terpenoids comprising 38 sesquiterpenoids, 58 monoterpenoids, one diterpenoid, two triterpenoids, and four more compounds in cannabis oil [6]. One source of terpenoids is leaves [7, 8] and flowers [9] of *Cannabis* [8, 10, 11]. *Cannabis* has a distinct odor due to chemicals called mono and sesquiterpenoids, while cannabinoids are odorless [12]. The distinct class of terpenophenolic secondary metabolites called cannabinoids is present in *Cannabis*

plants and is principally in charge of the biological activity of the plant. With over 500 constituents in *Cannabis*, cannabinoids are just one of many, alongside phenolics, flavonoids, terpenes, and alkaloids [13]. There have been 125 identified cannabinoids, categorized into 10 sub-groups based on their structures. These sub-groups containing cannabichromene (CBC), cannabigerol (CBG), cannabidiol (CBD), D9-tetrahydrocannabinol (D9-THC), cannabicyclol (CBL), cannabiniol (CBN), and others cannabinoids. The neutral form of cannabinoids has a 21-carbon atom skeleton, and the principal cannabinoids' structures are illustrated in Figure 1 [14].

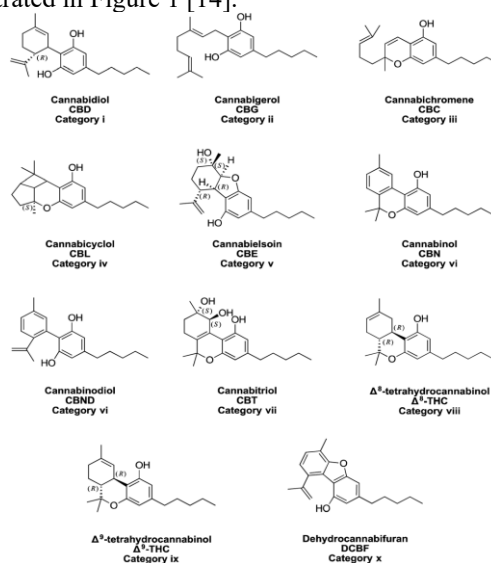


Fig. 1. Structures of principal cannabinoids.

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Nonetheless, a significant barrier to the extraction of *C. sativa*'s bioactive components is the plant's chemical complexity [15]. Because of this, it is essential to comprehend the proper and efficient methods for extracting and separating the bioactive components of *C. sativa* in order to investigate their chemical and biological activity, as well as to use them for therapeutic purposes and large-scale industrial production.

Currently, for novel industrial uses, the Moroccan government is thinking about legalizing and controlling the production of *Cannabis* with low THC content. Cultivating cannabis for nutritional, cosmetic, and medical uses is becoming more popular. Whole hemp seeds can be used in food, and hemp seed oil and flour can be made. The essential oil of the *C. sativa* plant is particularly beneficial in nutrition due to its components [16, 17].

As far as our study of the literature has been able to verify, no report has been published concerning the chemical composition and antioxidant capacity of *C. sativa* EO from the Al-Hoceima region. In this context, the aim of the present study is to define and analyze the chemical composition and evaluate the antioxidant activity of *C. sativa* EO plant for the first time in the province of Al Hoceima, and comparing the results obtained with other studies of the same plant in other regions, inside or outside of Morocco.

2 Experimental

2.1. Plant material

Cannabis sativa subsp. sativa L. aerial parts (Figure 2) were collected in the northern Moroccan province of Al Hoceima, about 467 kilometers away from Rabat, the country's administrative capital. Located on the Mediterranean coast, Senada is a part of the province of Al-Hoceima. After the territorial division in 2015, it became a member of the Tangier-Tetouan-Al Hoceima region.

The coordinates of the study area are 35°01'36.0"N, 4°11'44.1"W. It is distinguished by a landscape of mountains rising to an elevation of 1250–1450 meters and an abundance of flowers (Figure 3) [18].



Fig. 2. *C. sativa* from the Al-Hoceima province

This area renowned for its longstanding tradition of *Cannabis* cultivation and is considered one of the largest *Cannabis* producing regions in the world. The terroir, which includes factors such as soil, climate, and altitude, also contributes to the characteristics of the *Cannabis* compositions found in the Rif region. The plant was harvested in September 2021 after flowering. The voucher specimen (RM-01) has been deposited and well-preserved away from light at the department of Chemistry, Laboratory Research Unit in Applied Chemistry (RUAC), Faculty of Science and Technology Al Hoceima, Abdelmalek Essaadi University, Morocco.

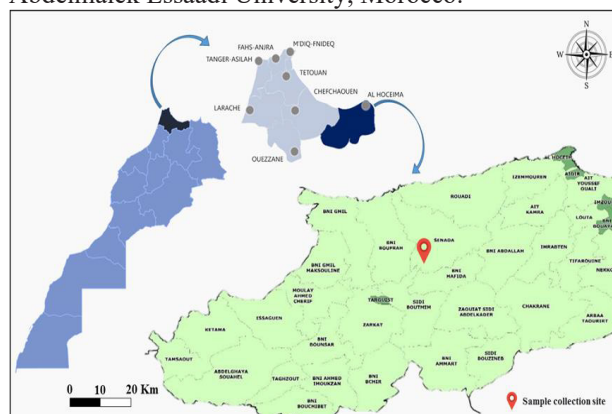


Fig. 3. Mapping representation of the sample collection site

2.2. Hydrodistillation process

Hydrodistillation is a widely utilized technique for obtaining bioactive substances from plants. Unlike steam distillation, which employs steam, hydrodistillation involves the use of water, steam, or a mixture of both to extract the compounds. This method is considered environmentally friendly since it doesn't use any organic solvents. It should be mentioned, that because of the high temperature needed, volatile and heat-sensitive compounds may be lost during the extraction process [19]. A Clevenger-style equipment was used to hydrodistillate the aerial components. A quantity of 100 g of the plant material was introduced into a flask filled with a sufficient amount of distilled water. The whole was then brought to a boil using a balloon heater set at a temperature in the region of 100 °C. The vapors generated after boiling are directed to a refrigerant where they will condense to form droplets of essential oil. The recovery of the EO was made after 3h of extraction. The EO obtained was then collected in vials and then stored in the refrigerator.

2.3. GC-MS Analysis of *C. sativa* EO

Essential oil of *C. sativa* was detected and separated using a Shimadzu GC system (Kyoto, Japan) equipped with a QP2010 MS linked to a BPX25 capillary column (30 m × 0.25 mm inner diameter × 0.25 μm film thickness) with a 5% diphenyl, 95% dimethylpolysiloxane phase. The carrier gas was 99.99 percent pure helium gas flowing at a steady 3 milliliters per minute. The injection, ion source, and interface temperatures were held constant at 250°C. For a duration of one minute, the column oven's

temperature program was set at 50°C, which was then raised to 250°C at a rate of 10°C per minute. The components of the sample were ionized in the EI mode at 70 eV. 40–300 m/z was the mass range that was scanned. A splitless mode (split ratio 90:1) was used to inject 1µL of EO diluted with a suitable solvent (hexane). By comparing the mass spectrum fragmentation patterns of compounds with those conserved on file at the National Institute of Standards and Technology (NIST), compounds were identified, and by correlating their retention durations with real standards. Data processing and collection were conducted using LabSolutions (version 2.5).

2.4. Antioxidant activity of *C. sativa* EO

The DPPH• (2,2-diphenylpicrylhydrazyl radical) technique was utilized to evaluate the essential oils made from *C. sativa* for their antioxidant potential. Briefly, the essential oils of *C. sativa* inflorescence were prepared in ethanol at various concentrations ranging from 0.078 to 5 mg/mL. Samples were prepared by adding 50µl of each stock ethanolic solution of EO to 1.95ml of DPPH• ethanolic solution (0.04g/L) [20].

Simultaneously, a negative control was prepared by combining 1.95mL of DPPH-ethanolic solution with 50µL of ethanol. Absorbance was measured against a blank prepared for each concentration at 517nm using a spectrophotometer (Rayleigh – UV1800 V/VIS) after thirty minutes incubation at dark room temperature. The solution of ascorbic acid, a common antioxidant, was used

as the positive control. Three tests were conducted for every concentration. Results were presented as an inhibition percentage (%I) calculated according to the formula (1) [21].

$$\%I = \frac{Abs(control) - Abs(sample)}{Abs(control)} \times 100 \quad (1)$$

%I : Percentage of sample free radical inhibition

Abs (control) : Absorbance of DPPH• measured alone, read at 517nm.

Abs (sample) : Absorbance of the sample in the presence of DPPH• read at 517nm.

The IC50 values were utilized to express the essential oils antioxidant ability. These values indicate the concentration of samples needed to scavenge 50% of the DPPH• radicals.

3 Results and discussion

3.1. Chemical composition and yields of *C. sativa* EO

The yield of extracting *C. sativa* essential oil was 0.23 ± 0.02% (w/w). This outcome is consistent with information provided in a recent study [22]. The whole composition and yield of extraction of the essential oil acquired from the inflorescences of *C. sativa* are presented in Figure 4 and Table 1. There was a total of 28 chemical components found in the EO extract, which represents 100% of the total content. Caryophyllene (31.77%), α-humulene (11.21%), β-myrcene (8.76%), were the main constituents in the EO extract.

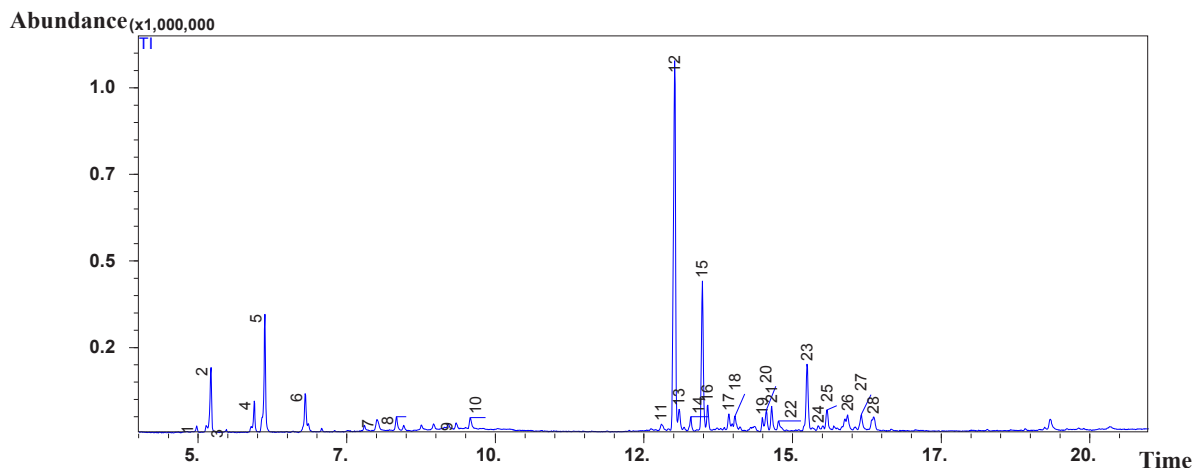


Fig. 4. Chromatogram of the volatile fraction of *C. sativa* examined by GC-MS

Table 1. Chemical profile and extraction yield of the volatile constituents extracted from *C. sativa*.

Peak number	Compounds	R.T	Area(%)
1	4(10)-Thujene	4.975	0.32
2	alpha.-Pinene	5.214	4.31
3	Camphene	5.475	0.14
4	beta.-Pinene	5.944	2.13
5	beta.-Myrcene	6.123	8.76
6	D-Limonene	6.803	3.30
7	Linalool	8.008	2.03
8	2-Norbornanol, 1,3,3-trimethyl	8.336	1.63
9	beta.-Citronellal	9.341	0.48
10	alpha.-Terpineol	9.577	2.01
11	Isocaryophyllene	12.794	1.19

12	Caryophyllene	13.018	31.77
13	alpha.-Bergamotene	13.093	1.90
14	beta.-Farnesene	13.288	1.41
15	alpha.-Humulene	13.482	11.21
16	(+)-Aromadendrene	13.572	2.18
17	beta.-Selinene	13.929	1.67
18	-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	14.031	1.54
19	alpha.-Bisabolene	14.493	1.06
20	beta.-Panasinsene	14.557	1.91
21	Valencen	14.648	2.29
22	(6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	14.765	1.36
23	Isoaromadendrene epoxide	15.244	6.22
24	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	15.429	0.24
25	2,5,9-Trimethylcycloundeca-4,8-dienone	15.579	2.25
26	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	15.924	1.77
27	Aromadendrene oxide-(1)	16.156	2.40
28	alpha.-Bisabolol	16.362	2.52
	Total (%)	100	
	extraction yield (% w/w)	0.23 ± 0.02	

R.T : Retention Time

Comparative analyses showed that our *C. sativa* EO from Al-Hoceima has a different chemical profile than prior reports from both inside and outside of Morocco. A study inside of Morocco reported that the chemical composition of *C. sativa* EO aerial parts taken from KETAMA in the Rif area (34°54'57"N, 4°34'07"W) was examined using GC/MS, and the results showed that α -humulene (12.8%), caryophyllene oxide (10.6%) and (E)-caryophyllene (35.0%) predominated [23]. Outside of Morocco, Benelli et al., found that myrcene (14.2%), α -pinene (16.4%) and (E)-caryophyllene (23.8%), were the primary volatile components of *C. sativa* EO from newly formed inflorescences of industrial hemp [24]. Another study revealed that myrcene (12.3–13.6%), (E)-caryophyllene (19.4–19.5%), α -pinene (20.3–20.4%) and terpinolene

(15.0–19.1%) were the main constituents of the essential oil recovered from *C. sativa* inflorescences grown in Italy [25]. Furthermore, a study revealed that the chemicals that predominated in five *C. sativa* cultivars farmed in Austria were (E)-caryophyllene (12.19–18.93%), myrcene (21.08–35.02%), α -terpinolene (7.02–16.61%), α -humulene (6.10–8.71%), α -pinene (7.21–14.61%) and (E)- β -ocimene (7.33–9.04%) [26]. According to these data, the composition of *C. sativa* EO appears to have been very variable within-species, which may be related to the growth environment of the plants. The content of the oil can really be influenced by a wide range of intrinsic (genetic features) and extrinsic (location, time of collection, age of plants, etc.) factors [27, 28]. Table 2 summarizes these studies about *C. sativa* EO.

Table 2. Comparison of different research about *C. sativa* EO.

<i>Cannabis</i> collection regions	Extraction method	Extraction time	Main components	Yield of extraction (% w/w)	Référence
Fiuminata, central Italy	Steam distillation	3h	- Myrcene (14.2%) - α -pinene (16.4%) - (E)-caryophyllene (23.8%)	0.3	[24]
Pisa, Italy	Hydrodistilled by a Clevenger apparatus	2h	- α -pinene (20.3–20.4%) - terpinolene (15.0–19.1%) - α -humulene (6.10–8.71%) - (E)-caryophyllene (19.4–19.5%)	0.11- 0.25	[25]
Amstetten, Austria	Hydrodistillation		- myrcene (12.3–13.6%). - (E) β -ocimene (7.33–9.04%) - α -pinene (7.21–14.61%) - myrcene (21.08–35.02%) - (E)-caryophyllene (12.19–18.93%)	-	[26]
Ketama, Morocco	Hydrodistillation using a Clevenger-type apparatus	3h	- α -terpinolene (7.02–16.61%) - (E)-caryophyllene (35.0%) - α -humulene (12.8%) - caryophylleneoxide (10.6%).	2.7	[23]
Al-Hoceima, Morocco	Hydrodistillation using a Clevenger-type apparatus	2.5h	- caryophyllene (31.77%) - α -humulene (11.21%) - β -myrcene (8.76%)	0.23	Our study

3.2. Antioxidant activity

Antioxidant capacity of the *C. sativa* essential oils was investigated using the DPPH assay to determine the inhibition concentration (IC₅₀), deduced from the curve that shows the percentage of inhibition in relation to the essential oil concentration (Figure 5).

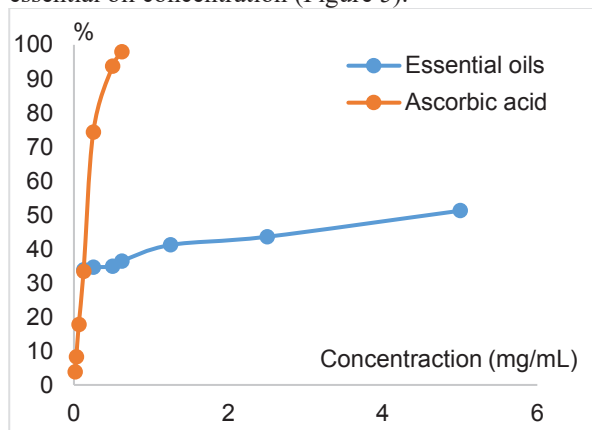


Fig. 5. Inhibition percentage profile of *C. sativa* essential oils

Table 3 displays the obtained IC₅₀ results. The data showed that the percentages of inhibition found are moderate, ranging from 33% to 51%. The IC₅₀ values of our essential oils (4.45 mg/mL) were much higher than that of ascorbic acid (0.16 mg/mL) and also higher than the IC₅₀ values (1.6 mg/mL) of *C. sativa* essential oils obtained in a recent study [23]. It can be stated that our *C. sativa* essential oils exhibit interesting antioxidant activity. The main reason for our EO's antioxidant efficacy could be its (E)-caryophyllene content. Indeed, it has been demonstrated in the past that species that are abundant in this chemical compound exhibit notable antioxidant action [29-31].

To our knowledge, the findings of this investigation represent the first data to be published on *C. sativa* EO from Al-Hoceima region concerning antioxidant activity. The obtained results indicated that (E)-caryophyllene and α -humulene, two sesquiterpene components, predominate in Moroccan *C. sativa* EO responsible for an important antioxidant activity of cannabis.

Table 3. IC₅₀ of *C. sativa* essential oils

Samples	IC ₅₀ (mg/mL)
Essential oils	4.45
Ascorbic acid	0.16
Essential oils [22]	1.6

4 Conclusion

This initial investigation reveals that the essential oil obtained from *C. sativa* plants grown in the Al-Hoceima region contains a considerable amount of phytochemicals associated with various therapeutic advantages. This versatile plant serves as a source of various bioactive components that can be used for innovative industrial applications in the fields of food, medicine, and cosmetics. The principal constituents of this essential oils are sesquiterpene compounds, such as caryophyllene, α -humulene, and β -myrcene. The chemical profile of *C.*

sativa EO cultivated in the Al-Hoceima region differs in comparison with other regions. The results showed also that our EO displayed interesting antioxidant activity. Further research is necessary to evaluate its potential antimicrobial and antifungal properties and recommend using it in the field of food processing.

Acknowledgments

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