Phytochemical Study and Antioxidant Potential of Three Leaf Extracts of *Cannabis sativa* L from the Al-Hoceima Region, Morocco

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Abstract. Within Morocco's efforts to increase the value of cannabis, we investigated the 'Beldeya' *cannabis sativa* L plant, which is produced in the Ketama area of northern Morocco. In this investigation, we performed phytochemical screening and quantitative analyses of ethanol, chloroform, and hexane extracts from cannabis leaves. Additionally, we explored the antioxidant potential using two extraction methods: Maceration and Soxhlet extraction. According to the results of the phytochemical screening, most metabolites were present in the extracts, except for alkaloids and anthraquinones which were not detected in any of the extracts. We employed standard methods for the quantitative measurement of total phenol (TPC) and flavonoid content (TFC), and the results showed that the TPC and TFC were abundantly present in the hexane extract using both maceration and Soxhlet extraction methods. Antioxidant potential was assessed through the Assay for reducing 2,2-diphenyl-1-picrylhydrazyl(DPPH). The findings demonstrate that the ethanolic extract shown greater activity for both extraction techniques, displaying an IC50 value of 222.96 µg/ml for Soxhlet extraction and 256.21 µg/ml for the maceration method. ***Keywords*:** Antioxidant properties, Polyphenol, Plant extract, *Cannabis sativa* L., Cannabis extracts

1 Introduction

*Cannabis sativa L.(*CS) is an annual plant belonging to the Cannabinaceae family, a multifunctional plant that is grown for its versatility and utilized in a variety of industries, including agriculture, paper manufacturing, textiles, biocomposites, biofuels, cosmetics, and construction [1]. CS, known locally in Morocco as "El kif", is widely cultivated in temperate climates and is found in northern Morocco (Rif region) [2].

One of the top cannabis producers in the world, Morocco, approved a measure on Thursday, March 11, 2021, allowing the plant to be used for medicinal, cosmetic, and industrial purposes. The goal of this legislation is to transform the usage of cannabis, which was illegal and harmful to the environment, into a sustainable legal industry that generates value and employment. With a regulatory framework, approved agricultural cooperatives, management of THC concentrations (the primary psychoactive component of cannabis), and certified plants, the bill enables the creation of the National Agency for the Regulation of Cannabis-Related Activities. This agency is responsible for developing the industrial agricultural cycle and managing the whole production process, from seed importation to marketing [3].

It is commonly recognized that CS leaves have euphoric, bitter, tonic, aphrodisiac, and analgesic qualities [4]. Sleeplessness, hysteria, skin diseases, diarrhea, and stomach problems are among the other conditions they are used to treat. Female inflorescences and cannabis leaves are covered with glandular trichomes, which are regarded as phytochemical biofactors [5].In general, the CS plant has rather high levels of polyphenols, flavonoids , terpenoids , sterols and cannabinoids [6]. According to Kornpointner *et al*. [7]There are about 500 phytochemicals in bark, flowers, roots, seeds, and leaves.

Giselle *et al*.[8]conducted a phytochemical and antioxidant investigation on hexanic extracts of the *Cannabis sativa* plant, revealing the presence of flavonoids and polyphenols, along with antioxidant activity. Prior investigations into the cannabis plant in the Ketama region have focused on the research of seeds, as demonstrated by S.Haddou *et al*.[9] and the study of essential oils, as investigated by El Bakali *et al*.[10]. However, research on cannabis leaf extracts in the northern Moroccan province of Ketama has not yet been conducted.

Studying the chemical profiles and biological activities of CS flowers grown in the Ketama region can help assess their medicinal potential and public health implications, thus contributing to the preservation of the

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plant's genetic diversity and the conservation of the local ecosystem.

To compare the outcomes with research conducted in other areas, the current study examined the phenolic components and antioxidant activity in leaves of CS grown in the Ketama region using two extraction techniques: maceration and Soxhlet extraction. It also serves as a resource for the plant's possible application in a variety of industries.

2 Materials and methods

2.1 Sampling procedure

In August 2023, the CS leaves were gathered at an altitude of 1100 meters in the northern Moroccan region of Ketama, situated in the center Rif (34°48'05.3"N 4°42'34.8"W). They were sliced with a unique chisel. The plant was identified by Brahim Ait Taleb, an agricultural engineer in the Department of Biology at the National Institute of Agricultural Research. Under voucher number CS02B/23, a herbarium sample was placed in the Faculty of Science and Techniques herbarium.

2.2 Preparation of samples

For a period of three weeks, the newly harvested leaves of CS were meticulously laid out to dry. After being ground in a grinding machine (Moulinex, France), the dried leaves were stored in glass vials that were kept out of the light and moisture in order to prepare them for further study.

2.3 Extract preparation

2.3.1Soxhlet's method

A cartridge containing 30 g of powdered leaves was subjected to ethanol, chloroform, and hexane. When the liquid around the cartridge turned colorless, asserting that solids were no longer escaping into the solvent. Subsequently, a rotary evaporator was employed to concentrate the solution after each extraction until a solid was obtained.

2.3.2 Maceration method

100 ml of ethanol, chloroform, and hexane were combined with 30 g of powder. The mixture was then manually shaken for 10 minutes to homogenize it. Next, a Büchner vacuum filter was used to filter the mixture, and the three filtrates were concentrated on a water bath.

2.4 Phytochemical screening

To identify compounds such as alkaloids, flavonoids, saponins, anthocyanins, sterols, triterpenes, and tannins in various plant extracts, phytochemical screenings are employed. These substances have distinct characterization reactions and are grouped based on their chemical affiliation. The characterization methods are based on those that Sofowora [11] and Haifa [12] have detailed. The reagents used in the chemical screening are listed in Table 1.

Table 1. Phytochemical screening reagents.

Chemical group	Identification reagents
Flavonoides	Hydrochloric acid; Ammonia; Zinc coppers
Saponins	Foaming
Sterols&triterpens	Concentrated sulfuric acid; Acetic anhydride
Anthocyanins	10% H_2SO_4, Ammonia
Alkaloids	Mayer reagent; Dragendorff reagent; Wagner reagent
Terpenoids	silver nitrate; ammonia
Anthraquinone	Liebermann-Burchard reagent:
Tannins	$FeCl_3$ diluted at 2%.
Reducing compounds	Fehling reagent

2.5 Total phenolic content (TPC)

With a few changes, the Folin-Ciocalteu method is employed to estimate the TPC of extracts, as described by Nekhla *et al* [13] and Labhar *et al* [14].0.5 ml of extract, 1 ml of 20% sodium carbonate (Na2CO3), and 1 ml of a 10-fold diluted Folin-Ciocalteu reagent are combined. After 60 minutes of dark incubation at room temperature, the combination is measured by spectroscopy at 760 nm and compared to a blank. Gallic acid at different concentrations (0- 0.06 mg/ml) is used to build a calibration curve that is produced under the same conditions as the sample in order to measure the amount of polyphenols. Gallic acid equivalent milligrams per gram of extract (mg EAG /gext) are used to report results.

2.6 Total flavonoid content (TFC)

The aluminum trichloride method was used to calculate the TFC using quercetin as a reference, as previously reported by O. Benamari *et al*. [15] , with a few modifications. Each diluted extract was divided into 10 milliliter test tubes, and 1 milliliter of aluminum trichloride (2% in methanol) was added along with a drop of acetic acid. After 40 minutes of room temperature incubation, the mixture's absorbance was determined at 415 nm in comparison to a blank made with 1 ml of methanol, 1 ml of AlCl3 (2%) and an acetic acid drip. Under the same circumstances, a quercetin-based standard range (0-300 µg/ml) was also created. The extracts' flavonoid content was reported in mg EQ/gext.

2.7 Antioxidant activity

The extract's antioxidant component donates a proton to a DPPH° free radical, which stabilizes the free radical and makes it into a DPPH molecule. The concentration of free radicals is lowered as a result. When the antioxidant that donates hydrogen is depleted, absorbance falls [16].

To measure the DPPH radical scavenging activity, we used the method employed by L. Mensor *et al*. [17] with a few modifications. The initial stock solutions (1000 µg/ml) were diluted to obtain final concentrations of 850, 700, 550, 400, 250, and 100 µg/ml, using ethanol as the solvent.1 milliliter of DPPH ethanol solution (0.04 mg/ml) was added, and the combination was allowed to react at room temperature. The absorbance readings were recorded at 518 nm after 30 minutes.

The following two metrics are used to evaluate the antioxidant activity:

Inhibition percentage:

$$I\% = [CA - EA/CA] \times 100 \quad (1)$$

With I%: Inhibition percentage; EA: Extract Absorbance; CA: Control Absorbance

IC50 value:

The concentration of antioxidant needed to lower the initial concentration of DPPH radical by 50% is referred to as the fifty percent DPPH free radical inhibitory concentration (IC50) or EC50 for Efficient Concentration 50%. Antioxidant capacity is negatively correlated with it: a substance's IC50 value decreases with increasing antioxidant activity value [18].Using GraphPad Prism 10 software, the IC50 values are visualized by linear regressions of the percentages of DPPH inhibition against various concentrations of the extracts under test.

3. Results and discussion

3.1 Extraction efficiency

Based on the powder's total weight, the extraction yield was determined. Figure 1 displays the results that were obtained. The soxhlet extraction technique gives a higher yield than the maceration technique. The results indicated that for both extraction procedures, the highest yield was found in ethanol, which was followed by hexane and chloroform. These results are explained by changes in the solvent's polarity. The extraction yield grows with increasing solvent polarity.

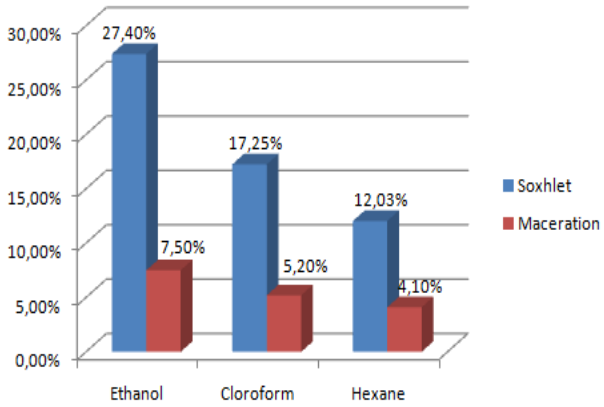

Fig. 1. Extraction efficiency ($p<0.05$)

3.2 Phytochemical screening

A summary of the phytochemical screening results is provided in **Erreur ! Source du renvoi introuvable.**.The main and secondary metabolites are present in every extract, as the table indicates. Nevertheless, anthraquinones and alkaloids are not.

Table 2: phytochemical screening results of three leaf extracts of C.S.

	Soxhlet			Maceration		
Chimical	Et.E	Ch.E	He.E	Et.E	Ch.E	He.E
Flavonois	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Sterols&tri-terpens	+	+	+	+	+	+
Anthocynins	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Anthraqui-none	-	-	-	-	-	-
Tannins	+	+	+	+	+	+
Reducing-compounds	+	+	+	+	+	+

Note: - : absent + : present ; Et.E: extract of ethanol,Ch.E:extract of chloroform ,He.E: extract of hexane

3.3 Determination of total polyphenols

By extrapolating from the calibration curve ($Y= 8.59X–0.022$; $R2 = 0.998$), the three extracts' TPC was determined, and it was given as mg GAE/g_{ext}. **Erreur ! Source du renvoi introuvable.**displays the results for each of the three extracts. The TPC of the various fractions varied from 0.615 to 2.213 mg. Using both extraction methods, the hexanic extract contained the highest amount of polyphenols (2.213 mg EAG /g_{ext}).Galanakis *et al*. [19] provide an explanation for the results, demonstrating that phenols' solubility in various solvents is not only reliant on their polarity. The solubility propensity can be accounted for by the intermolecular interactions and stereochemistry of phenols, particularly hydrogen bonds between solvents and phenols.

Drinićetal's results [20], which show that ethanolic extract contains 5.85 mg EAG/gext of polyphenols, are not comparable to our result, The genotype of the plant or external circumstances might be the cause of this variation [21].

Table 3. Polyphenol assay results

Chemicals	**Total phenol (mg EAG / g_{ext})**	
	Soxhlet	**Maceration**
Hexane	2.213	1.123
Chloroform	1.790	0.615
Ethanol	0.823	0.961

3.4 Determination of total flavonoids

Using quercetin as the reference standard, the quantity of flavonoids in CS leaf extracts was measured using a calibration curve (Y=1.553x – 0.007; R2 = 0.998). Quercetin equivalents (EqQ) are used as the unit of measurement. Table 4 displays the results, which indicate that the extraction process and solvent utilized have a significant impact on the overall flavonoid content. With 0.421 mg QE/g_{ext} for the soxhlet technique and 0.425mg QE/g_{ext} for the maceration technique, the hexane extract has the greatest flavonoid concentration. The findings of Jin *et al* [6], who showed flavonoid quantities in CS leaves ranging from 0.34% to 0.44%, are in line with these results.

Table 4. Flavonoid assay results

Chemicals	Total flavonoids (mg EQ / g_{ext})	
	Soxhlet	Maceration
Hexane	0.421	0.245
Chloroform	0.249	0.132
Ethanol	0.272	0.125

3.5 Antioxidant activity

The results of the antioxidant activity against the DDPH radical of several *C. sativa* L extracts and the reference are shown in Figures 2 and 3. The DPPH radical was significantly decreased by the leaf extracts in a dose-dependent manner at all investigated doses for both extraction methods, ethanolic extract exhibited more activity than hexanic and chloroformic extracts. The ascorbic acid employed as a reference demonstrated strong antioxidant activity at all tested doses when compared to the extracts. IC50 values were calculated (Figure 4) to enable a comparison of the activities among the various extracts from the tested plant's leaves. The standard provides an IC50 value of 72.88 µg/ml. The highest IC50 values were obtained by chloroform, with 741.6 µg/ml for the Soxhlet technique and 826.33 µg/ml for the maceration extraction. The ethanolic extract showcases IC50 values of 222.96 µg/ml for the Soxhlet technique and 256.21 µg/ml for the maceration extraction, while the hexanic extract presents IC50 values of 353.67 µg/ml for the Soxhlet technique and 455.28 µg/ml for the maceration extraction.The findings suggest that ascorbic acid exhibits greater DPPH radical scavenging activity compared to all three extracts being studied.

Sokół-Łętowska et al [22] state that phenolic compounds are appealing candidates for antioxidant activity because they contain several hydroxyls that may interact with free radicals. This theory does not align with our findings. Despite the hexanic extract's greater concentration of flavonoids and polyphenols, the ethanolic extract had more antioxidant action. The work by J. Chen *et al* [23] provides an explanation for this, arguing that the hydroxyl group structure of these compounds plays a major role in their ability to capture free radicals.

In comparison with other regions, the antioxidant activity found by [24] in the hexanic extract of Cannabis sativa leaves grown in Fiuminata, central Italy, is different, showing an IC50 of around 0,097mg/ml.

Antioxidant activity results found by N. Ahidar [25]show an IC50 of 0,231mg/ml for the ethanolic extract in cannabis flowers harvested from the same region.

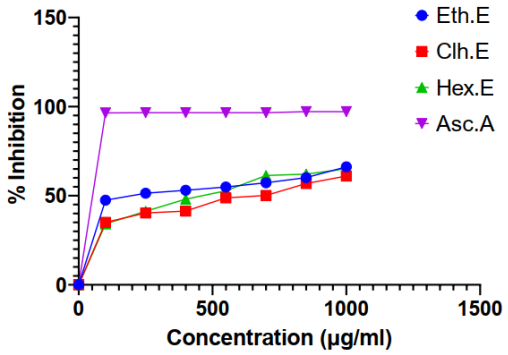

Fig. 2. DPPH radical scavenging capacity (Inhibition %) for Soxhlet extraction.

Note:Hex.E: hexan extract , Clh.E : chloroform extract, Eth.E: ethanol extract , Asc.A : ascorbic acid.

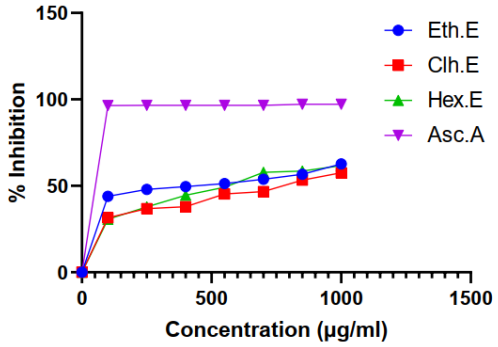

Fig. 3. DPPH radical scavenging capacity (Inhibition %) for Maceration extraction.

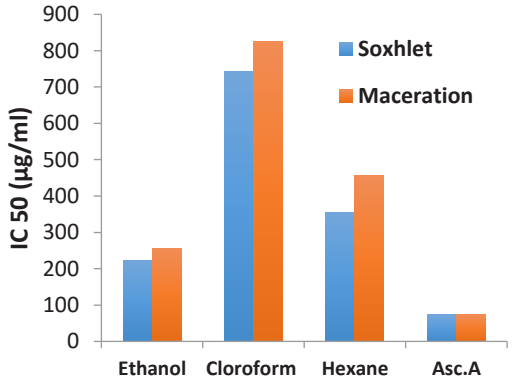

Fig. 4. IC50 values for extracts and ascorbic acid

4. Conclusion:

In the Rif area of northern Morocco, cannabis is an old plant. Studies on this plant have expanded to demonstrate its advantages since cannabis was legalized in this country. Here, our focus was on analyzing the phytochemical composition and evaluating the antioxidant capacity of several leaf extracts from CS plants that were collected in the Ketama area through the use of two extraction techniques (maceration and Soxhlet extraction). Secondary metabolites are abundant in our plant, as shown by the phytochemical screening carried out employing characterisation methods. Utilizing three different solvents for the extraction of the different secondary chemicals allowed us to determine the yield of each extract. The phenolic components and flavonoids with highest concentration were found in the hexanic extract.

According to studies on the antioxidant capacity of CS leaf extract employing the DPPH radical scavenging method, the ethanolic extract exhibits significant antioxidant activity. Its activity is still far less than that of ascorbic acid, though. It is imperative to bear in mind that the extract is essentially a crude blend of many different substances. Therefore, if developed, these molecules should behave similarly to ascorbic acid.

“We are thankful to department of chemistry, Faculty of Science and Technology, Abdelmalek Essaadi University. Al Hoceima, Morocco.”

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