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Phenolic Content and Antioxidant Activity of *Cannabis sativa L.* Flowers from the Ketama Region in Northern Morocco

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ABSTRACT

Within the framework of Bill No.13-21 on the legal use of cannabis in Morocco, we studied the *Cannabis sativa L.* plant, known as 'Beldeya,' grown in the Ketama region of northern Morocco. In the present study, phytochemical screening, a quantitative study on ethanol, chloroform, and hexane extracts of cannabis flowers, and a study of antioxidant potential were carried out. In all three extracts, phytochemical analysis revealed the presence of different kinds of secondary metabolites, including flavonoids, terpenoids, saponins, anthocyanins, tannins, and reducing sugars. The Folin-Ciocalteu technique was used to measure the total amount of polyphenols, and the results showed that the total amount of polyphenols varied from 1.802 mg to 2.225 mg of gallic acid equivalent (EqAG). Flavonoid assay with Aluminum chloride ($AlCl_3$) revealed that all three extracts contained flavonoids, ranging from 0.242 mg to 0.442 mg quercetin equivalent (EqQ). Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) reduction test, antioxidant activity was evaluated. The results obtained show that the ethanolic extract exhibited superior activity, with an IC50 value of 231.39 µg/ml, followed by the hexanic and chloroformic extracts, having IC50 values of 376.40 µg/ml and 769.60 µg/ml, respectively.

Keywords: *Cannabis sativa* L; plant extract; antioxidant activity; phenolic content; flavonoid content.

INTRODUCTION

Cannabis sativa L. (CS) is an annual plant from Asia belonging to the Cannabaceae family (Bonini et al., 2018). It is a versatile and multifunctional crop that supplies raw materials for various industrial applications, encompassing traditional and innovative uses(Amaducci et al., 2015). It has been incorporated into several agro-industrial sectors, including agriculture, textiles, biocomposites, construction, automotive, paper, biofuels, functional foods, oils, cosmetics, personal care, and medicines (Salentijn et al., 2015).

The cannabis plant initially appeared in the Maghreb region in the seventh century, during the Arab conquests of North Africa, even before it was established in the Ketama region of the Sanhaja region, in the center of the Rif, in Morocco, in the fifteenth century. Over the past 40 years, cannabis has grown from these traditional producing regions to Taounate and Laarache in the south and west (Haddou et al., 2023).

Ethnobotanical research carried out in the Rif region revealed that cannabis was used as a medicinal plant to treat a variety of ailments, such as abortion, respiratory ailments, intestinal parasites, nausea, wounds, infantile diarrhea, hair care, vision problems, and ear infections (Merzouki et Molero mesa, 1999 and Clarke et Merlin, 2016).

In March 2021, Morocco adopted Bill No. 13-21, authorizing the medical, cosmetic, and industrial use of cannabis, paving the way for the legalization of its therapeutic use on its territory. The bill developed by the Ministry of the Interior regulates activities related to the cultivation of cannabis, its production, manufacture, transport,

and marketing, as well as its export and import for medical and therapeutic purposes, all by being subject to authorizations exclusively issued by a specialized Agency under the name of The National Agency for the Regulation of Activities Related to Cannabis (Highlights of Morocco's Bill N° 13-21 on the Legal Use of Cannabis, 2021). A phytochemical and antioxidant analysis carried out by (Giselle et al., 2023) on hexanic extracts of cannabis sativa flowers shows the presence of polyphenols and flavonoids, as well as antioxidant activity.

Generally speaking, flavonoids, polyphenols, cannabinoids, terpenoids, and sterols are relatively abundant throughout the CS plant (Jin et al., 2020). There are more than 500 phytochemicals that have been found in flowers, leaves, seeds, bark, and roots(Kornpointner et al., 2021). Previous scientific studies carried out on the cannabis plant in the Ketama region of northern Morocco concern the study of seeds, such as that carried out by Haddou et al. (2023), and a study of essential oils which was conducted by El Bakali et al. (2022). However, to date, no studies have been carried out on cannabis flower extracts in the Ketama region of northern Morocco.

The present study investigated the phenolic compounds and the activity of the antioxidant in various solvents polarity on flowers of Cannabis sativa cultivated in the Ketama region of northern Morocco to compare them with other studies in different regions. In addition, it serves as a reference for future research on cannabis flower extracts in the Ketama region and provides valuable information for their valorization in various fields.

MATERIALS AND METHODS

Collection of samples

CS flowers were harvested in July 2022 in Ketama region of northern Morocco, in the central Rif (34°48'54.1 "N 4°42'11.5 "W), at an altitude of 1600 m. They were cut with a special chisel. Agricultural engineer Brahim Ait Taleb identified the plant at the National Institute of Agricultural Research's Department of Biology.

Preparation of samples

The fresh flowers of CS were carefully spread out for drying for three weeks. In order to prepare the dried flowers for future analysis, they were first ground in a grinder (Moulinex, France) and then kept in glass vials shielded from light and moisture.

Preparation of extracts

The CS extracts were obtained using the Soxhlet method. An amount of 30 g of flower powder was put into a cartridge, which was subsequently exposed to hexane, chloroform, and ethanol in increasing order of polarity. The solvent stopped removing solid compounds when the liquid surrounding the cartridge became colorless, signaling that it was no longer eluding solid substances. Following each extraction, a rotary evaporator concentrated the solution until a solid was achieved.

Phytochemical screening

In the various plant extracts, simple chemical assays are used in essential phytochemical screening to look for substances like flavonoids, alkaloids, anthocyanins, tannins, sterols, triterpenes, and saponins. These compounds are classified according to their chemical affiliation, and each has its characterization reaction. The characterization methods used are derived from those described by Sofowora. (1996) and Yadav et al. (2014).

Total phenol content (TPC)

The Folin-Ciocalteu technique, as reported by Nekhla et al. (2023), is used to estimate the TPC of extracts with a few modifications. In this procedure, 0.5 ml of extract is mixed with 1 ml of 10-fold diluted Folin-Ciocalteu reagent and 1 ml of 20% sodium carbonate (Na_2CO_3). The mixture is incubated in the dark at room temperature for 60 minutes, followed by a spectrophotometric reading at 760 nm in comparison with a blank. Polyphenols are quantified in accordance with a calibration curve created under identical circumstances to the sample using gallic acid at various concentrations ranging from 0 to 0.06 mg/ml. Results are given in milligrams of gallic acid equivalent per gram of extract.

Total flavonoid content

The protocol used was inspired by Zhishen et al. (1999) and Farooq et al. (2021). 400 µl of the extract and 120 µl of 5% $NaNO_2$ was combined in a glass hemolysis tube. 120 µl of 10% $AlCl_3$ were added after 5 minutes, and the mixture was

rapidly agitated. A volume of 800 µl of 1 M NaOHwas added to the medium after 6 minutes. At 510 nm, the absorbance is instantly measured and compared to the control. Quercetin has been dissolved in methanol. The concentrations of flavonoids were deduced from the range of the calibration curve established with quercetin at various concentrations ranging from 0 to 0.3 mg/ml

Antioxidant activity

In the presence of a DPPH° free radical, a proton from the antioxidant compound in the extract is transferred to the free radical, transforming it into a stable DPPH molecule. This reduces the concentration of the free radical. This decreases absorbance until the hydrogen-donating antioxidant is exhausted (Laib and Barkat, 2011). With few modifications to the procedure outlined by Louli et al. (2004) and Labhar et al. (2023), DPPH radical scavenging activity was assessed by dissolving 4 mg of DPPH in 100 ml of methanol to create a DPPH solution. The mixture was then left in the dark for three hours. To obtain the following concentrations (0, 100, 250, 400, 550, 700, 850, and 1000 µg/ml), a series of dilutions of the extract and the positive control, ascorbic acid, were prepared in glass tubes. 5 ml of the DPPH solution are then added. The tubes are shaken, then left at room temperature for 30 minutes in complete darkness. The negative control contains only the DPPH solution and methanol in equal volumes of 2.5 ml. A UV/Vis spectrophotometer is used to measure the absorbance at 515 nm to get the reading. The antioxidant activity is evaluated using the following two parameters:

Inhibition percentage

The following formula is used to get the inhibition percentage IP:

$$IP\,\% = [CA - EA/CA] \times 100 \qquad (1)$$

where: CA – control absorbance; EA – extract absorbance

IC50 parameter

The IC50, or the inhibitory concentration at 50% of the DPPH free radical (also referred to as EC50 for Efficient Concentration 50%), is defined as the antioxidant concentration required to reduce the initial concentration of DPPH radical by 50%. It is inversely related to antioxidant capacity: the stronger the antioxidant activity of a substance, the lower the IC50 value (Kadri et al., 2011). The IC50 values are determined graphically from linear regressions of DPPH inhibition percentages against different concentrations of the tested extracts using the GraphPad Prism 10 software.

RESULTS AND DISCUSSION

The extraction yields

Soxhlet extraction of *CS* flower powder using three solvents showed that ethanol has the highest yield, 29.83%, followed by Chloroform and hexane, which yielded around 26.23% and 14.10%, respectively (Figure 1). The variation in solvent polarity can be used to explain these findings. When the solvent's polarity increases, the extraction yield rises.

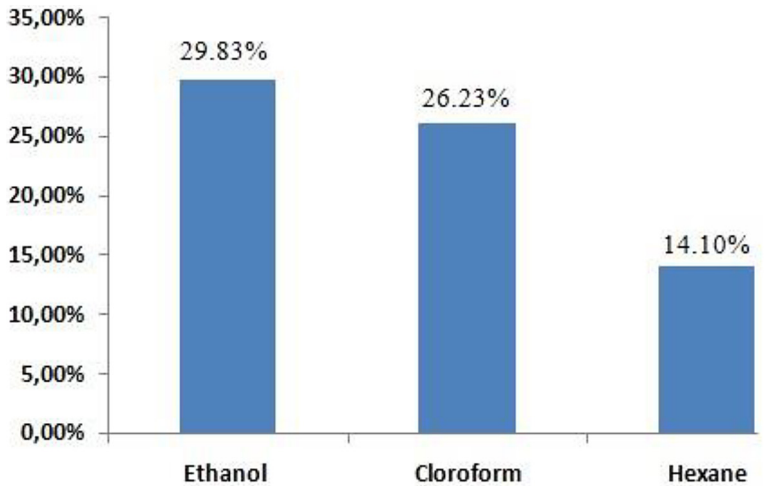

Figure 1. Extraction yield ($p<0.05$)

Phytochemical screening:

Table 1 presents the findings of the phytochemical screening. The primary and secondary metabolites are present in all extracts, as shown in the table. However, alkaloids and anthraquinones are not.

Determination of total polyphenols

The three extracts' TPC was calculated and represented as mg of gallic acid equivalent per gram of extract (mg GAE/g_{ext}). The gallic acid standard solution calibration curve was used to calculate the polyphenol concentration (Figure 2). Table 2 presents the outcomes for the three extracts. Regarding gallic acid equivalent (EqAG), the TPC of the different fractions ranged from 1.802 to 2.225 mg. The hexanic extract had the most significant polyphenols (2.225±0.01 mg EAG /g_{ext}). The findings are explained by Galanakis et al. (2013),who shows that phenols' solubility in different solvents cannot be entirely dependent on their polarity. The stereochemistry of phenols and intermolecular interactions, especially hydrogen bonds between phenols and solvents, can be used to explain the solubility tendency. These results are not similar to those of Giselle et al. (2023), indicating that hexanic extract has 101.98 mg EAG/ g_{ext} of polyphenols. This difference may be due to the plant's genotype or environmental factors(Pacheco-Hernández et al. 2021).

Determination of total flavonoids

A calibration curve was constructed using quercetin as the reference standard for determining the flavonoid content of *Cannabis sativa* L flower extracts (Figure 3). The unit of measurement is quercetin equivalents (EqQ). The results presented in Table 3 shows that total flavonoid content varies considerably depending on the extraction solvent used. The hexanic extract has the highest flavonoid content, with 0.442 mg quercetin equivalent per gram extract (mg QE/g_{ext}), followed by the ethanolic extract, which has a 0.267 mg QE/ g_{ext}. In contrast, the chloroformic extract has a content of 0.242 mg QE. These results are consistent with those found by Jin *et al*. (2020), who reported a flavonoid quantity ranging from 0.07% to 0.14% in *Cannabis sativa* L flowers.

Antioxidant activity

Figure 4 displays the findings of the antioxidant activity against the DDPH radical of several *CS* extracts and the reference. At all tested concentrations, the flower extracts significantly

Table 2. Amount of total phenol in the extracts

Chemicals	Total phenol (mg EAG/g_{ext})
Ethanol	1.938±0.01
Chloroform	1.802±0.01
Hexane	2.225±0.01

Note: the values presented are expressed as mean ± SD (n=3).

Table 3. Amount of flavonoids in the extracts

Chemicals	Total flavonoids (mg EQ/g_{ext})
Ethanol	0.267±0.01
Chloroform	0.242±0.02
Hexane	0.442±0.01

Note: the values presented are expressed as mean ± SD (n=3).

Table 1. Phytochemical constituents of three solvent extracts of Cannabis sativa L flowers

Chemical	Ethanolic extract	Chloroformic extract	Hexanic extract
Alkaloids	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Anthocyanins	+	+	+
Anthraquinone	-	-	-
Terpenoids	+	+	+
Sterols&triterpenes	+	+	+
Reducing compounds	+	+	+
Saponins	+	+	+

Note: + present, - absent.

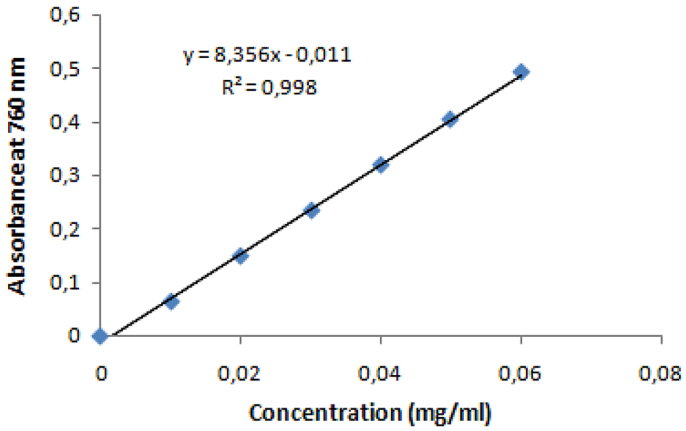

Figure 2. Gallic acid standard curve($p<0.05$)

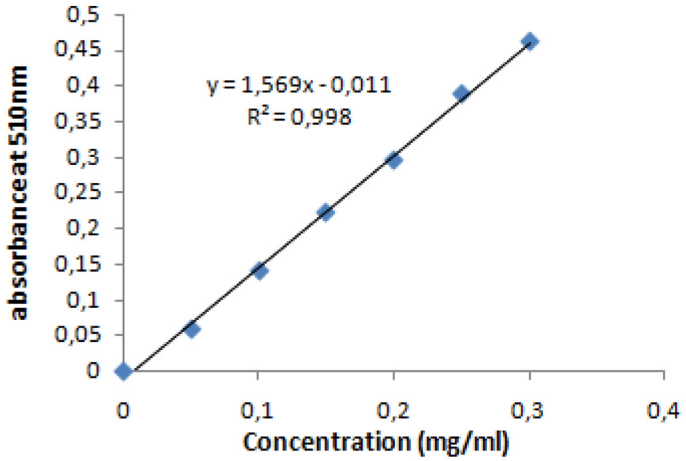

Figure 3. Quercetine standard curve ($p<0.05$)

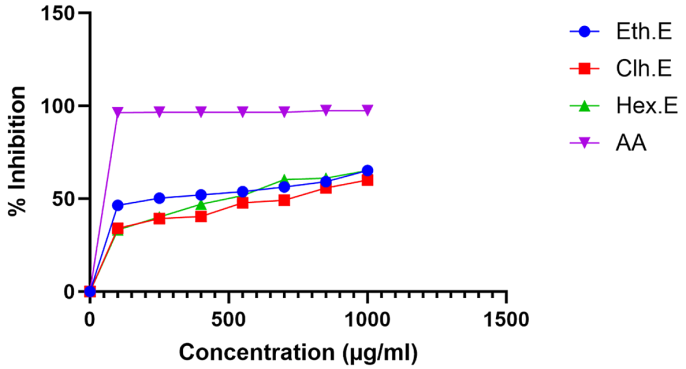

Figure 4. DPPH radical scavenging capacity (Inhibition %) ($p<0.05$). Note: Eth.E – ethanol extract; Clh.E – chloroform extract; Hex.E – hexan extract; (AA) acid ascorbic.

inhibited the DPPH radical in a dose-dependent manner. Ethanolic extract showed higher activity than chloroformic and hexanic extracts. Compared with the extracts, the ascorbic acid used as a reference showed powerful antioxidant activity at all the concentrations tested. To facilitate a comparison of activities between the different extracts from the flowers of the plant tested, IC50 values were determined (Figure 5). The IC50 value of the standard is around 73 µg/ml. Ethanolic extract stands out with an IC50 of 231.39 µg/ml, followed by hexanic extract and

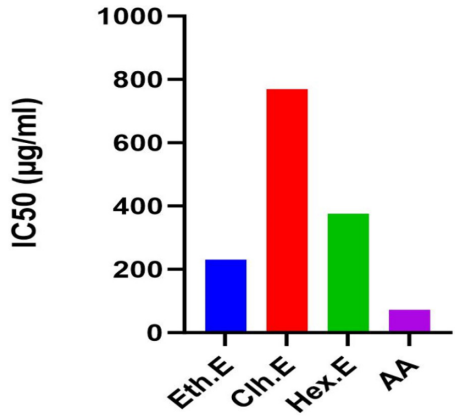

Figure 5. IC50 for extracts and ascorbic acid ($p<0.05$)

chloroform extract with IC50 values of 376.40 µg/ml and 769.60 µg/ml, respectively. The results show that all three extracts have a lower DPPH radical scavenging activity than ascorbic acid. According to Sokół-Łętowska et al. (2007). Phenolic substances have many hydroxyls that may interact with free radicals, making them attractive candidates for antioxidant action. Our results are not consistent with this hypothesis. The ethanolic extract exhibits higher antioxidant activity than the hexanic extract despite the richness of the latter in polyphenols and flavonoids. This can be explained by the study conducted by (Chen et al. 2020), which contends that these compounds' capacity to snare free radicals is mainly influenced by the hydroxyl group structure they contain.

CONCLUSIONS

Cannabis is an ancient plant in the Rif region, northern Morocco. After the legalization of cannabis in this country, studies on this plant have multiplied to confirm its benefits. In this context, we focused on the phytochemical study and the evaluation of the antioxidant capacity of various extracts from *CS* flowers harvested in the Ketama region. The phytochemical screening conducted using characterization reactions reveals our plant's abundance of secondary metabolites. Extraction of the various secondary compounds using three solvents enabled us to calculate the yield of each extract. The highest concentration of phenolic compounds and flavonoids was obtained in the hexanic extract. The ethanolic extract has substantial antioxidant activity, according to research on the antioxidant activity of *Cannabis sativa L* flower extract using the DPPH free-radical scavenging technique. However, compared to ascorbic acid, its activity is still much lower. It is critical to remember that the extract is a rough combination of several diverse chemicals. As a result, these molecules will likely display action similar to ascorbic acid once refined.

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