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Effect of solvent polarity on phenolic antioxidant extraction from *Cannabis sativa* L. leaves and inflorescences: mixture design optimization and HPLC-DAD/ESI-MS^2 profiling

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Cannabis (*Cannabis sativa* L.) is a source of bioactive compounds with promising applications in the nutraceutical, pharmaceutical, and cosmetic fields. Applying a mixture design approach with three solvents (water, acetone and ethanol), this study investigated how solvent composition modulates the recovery of phenolic compounds from cannabis inflorescences and leaves. Extracts were assessed for total phenolic content (TPC) and antioxidant activities using DPPH, ABTS, FRAP, CUPRAC, and TAC assays, and their chemical profiles were characterized by HPLC-DAD/ESI-MS^2. Solvent composition significantly affected extraction efficiency across plant organs. For inflorescences, the highest TPC value (16.05 mg GAE per g dry weight) was obtained with an acetone-enriched mixture (1/6 water–1/6 ethanol–2/3 acetone), whereas leaves reached their maximum (13.65 mg GAE per g DW) with 1/3 ethanol–2/3 acetone. Multi-response optimization identified 1/3 water–1/3 ethanol–1/3 acetone for inflorescences and 50% water–30% ethanol–20% acetone for leaves as the optimal solvent mixtures. HPLC-DAD/ESI-MS^2 analysis identified 32 compounds belonging to several classes, including phenolic acids, flavonoids, hydroxycinnamic acid amides, lignanamides, and cannabinoid derivatives. These classes showed diverse content depending on the solvent and plant matrix. The acetone rich mixture produced the highest total cannabinoids (10.56 and 8.45 mg CBN equivalents per g dry weight) for inflorescences and leaves, respectively. These results reveal clear organ and solvent-dependent selectivity in Moroccan *Cannabis*. These differences were reflected in the antioxidant activities, with extracts showing notable reducing and radical-scavenging capacities across DPPH, ABTS, FRAP, CUPRAC, and TAC assays. These findings support the targeted valorization of cannabis leaves and inflorescences as sources of antioxidant phytochemicals for pharmaceutical and cosmetic applications.

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1 Introduction

Cannabis (*Cannabis sativa* L.) is an herbaceous plant belonging to the Cannabaceae family that has been grown all over the world for many different applications, including textiles, medicine, cosmetics, nutrition, and recreational purposes. Due to its many natural components, cannabis is a chemically complex species. Cannabinoids are the most distinctive class of cannabis metabolites and are mainly produced in glandular trichomes. The primary psychoactive compound, Δ9-tetrahydrocannabinol (Δ9-THC), naturally occurs in the plant as its acidic precursor, Δ9-tetrahydrocannabinolic acid (Δ9-THCA). It is formed by decarboxylation of Δ9-THCA during heating, processing, or ageing. Δ9-THC and its acidic precursor are among the most extensively investigated phytocannabinoids because of their psychoactive and medicinal relevance, whereas cannabidiol (CBD) and cannabidiolic acid (CBDA) are generally regarded as non-narcotic cannabinoids in cannabis.[1] In addition, other important cannabinoids reported in *Cannabis sativa* include tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC).[1,2]

In addition to cannabinoids, terpenes, flavonoids, stilbenoids, alkaloids, and other polyphenols are among the more than 500 compounds that contribute to the highly diverse phytochemical profile of *Cannabis sativa* L. Terpenes constitute a major class of non-cannabinoid compounds, with more than 100 identified molecules that are accumulated and produced in the glandular trichomes of female inflorescences, especially in

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the unfertilized ones, making this plant part the most valuable for the pharmaceutical sector.[3,4] Leaves also contain various bioactive compounds, including phenolic compounds such as flavonoids, cannabinoids, and other naturally occurring metabolites which have been reported to exhibit antibacterial and antioxidant activities.[5] Phenolic compounds are of particular interest because their hydroxylated structures confer reducing and radical-scavenging properties, thereby contributing to plant protection against oxidative stress caused by reactive oxygen species. The recovery of these compounds depends on several parameters, such as temperature, pH, particle size, liquid-to-solid ratio, and solvent composition, which is one of the most determining factors for isolating plant bioactive compounds because of their distinct chemical properties and polarities. Therefore, the solvent combination is the most successful method to improve the selectivity of antioxidant compound extraction.[6,7] Mixture-design strategies are effective within this framework to evaluate interactions between solvent components and to identify solvent proportions that maximize the recovery of bioactive compounds. In recent studies, mixture designs have been successfully applied to optimize the extraction of phenolic and other bioactive compounds from complex plant matrices.[8,9]

Although the cannabinoid profile of *Cannabis sativa* L. has been widely examined, the detailed study of its non-cannabinoid phenolic fraction has received far less attention, particularly in Moroccan genetic resources. In our earlier research, HPLC-DAD/ESI-MS^2 analyses showed that Moroccan *Cannabis* seeds are rich in phenolic constituents, with hydroxycinnamic acid amides and lignanamides as the major classes.[7] However, seeds, represent only one type of plant matrix, while leaves and inflorescences are distinct organs with their own chemical and physiological features. Female inflorescences contain numerous glandular trichomes and are therefore expected to accumulate higher levels of cannabinoid derivatives, whereas leaves may be an underused source of polar phenolics, including flavonoids and hydroxycinnamic acid amides. To our knowledge, no study has yet offered a detailed HPLC-DAD/ESI-MS^2-based characterization of phenolic and related terpenophenolic compounds in both leaves and female inflorescences of the Moroccan *Cannabis sativa* L. Beldia ecotype.

In this study, we hypothesized that the phytochemical profile and antioxidant capacities of Moroccan *Cannabis sativa* L. Beldia vary significantly depending on the plant organ, and the polarity of the solvent influences the extraction of different classes of antioxidant compounds from both leaves and inflorescences. Specifically, we expected that mixtures rich in organic solvents would be more effective at extracting less polar compounds, such as cannabinoid derivatives, particularly from inflorescences, whereas water-containing mixtures would enhance the recovery of more polar phenolic compounds, especially from leaves. Therefore, the present study aimed to investigate the effect of water–ethanol–acetone mixtures on the extraction of antioxidant compounds from *Cannabis sativa* L. leaves and inflorescences using a simplex lattice mixture design. The resulting extracts were evaluated for total phenolic content and antioxidant activity through complementary assays, and their phytochemical profiles were examined through HPLC-DAD/ESI-MS^2 allowing us to establish connections between solvent composition, the specific plant organ, chemical profile, and antioxidant capacity.

2 Materials and methods

2.1 Chemicals and reagents

Ethanol, acetone, and water (analytical grade) were used for extraction, whereas acetonitrile and formic acid (LC-MS grade) were used for chromatographic analysis. Cannabinol (CBN), *N-trans*-caffeoyltyramine, ferulic acid, and quercetin were used as analytical standards for cannabinoids, hydroxycinnamic acid amides, phenolic acids, and flavonoids, respectively. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 1 Experimental design and responses for the solvent mixture system for inflorescences and leaves of *Cannabis sativa* L.[a]

Independent variables				Responses											
Run	Water	Ethanol	Acetone	TPC-L	TPC-I	TAC-L	TAC-I	DPPH-L	DPPH-I	ABTS-L	ABTS-I	FRAP-L	FRAP-I	CUPRAC-L	CUPRAC-I
1	1/3	2/3	0	10.55	12.42	15.70	15.69	4.27	4.90	8.48	8.45	51.06	33.24	3.37	18.13
2	2/3	1/3	0	12.00	8.58	7.13	9.68	3.98	4.80	7.83	8.68	42.74	35.05	5.96	17.54
3	1	0	0	12.79	5.98	9.00	14.50	4.69	3.12	7.27	7.69	43.73	39.90	6.15	16.24
4	2/3	1/6	1/6	12.80	9.50	11.06	11.11	5.78	4.77	8.60	8.65	51.55	45.78	7.89	7.68
5	1/6	1/6	2/3	11.10	16.05	7.63	10.81	3.78	4.64	7.71	8.35	39.60	52.79	11.50	10.49
6	0	1	0	11.81	12.36	11.21	12.12	5.26	4.45	8.64	7.90	27.41	21.06	8.77	9.87
7	0	0	1	9.34	9.97	29.03	3.53	3.72	4.37	8.10	7.16	50.88	16.38	6.88	7.27
8	1/3	1/3	1/3	12.03	13.86	9.52	14.45	4.83	5.11	8.85	8.54	46.55	48.98	10.05	18.08
9	0	1/3	2/3	13.65	12.81	11.27	8.91	5.77	4.27	8.56	7.31	43.15	23.69	14.90	15.20
10	1/3	0	2/3	9.80	14.67	31.73	12.88	4.8	5.08	8.12	8.52	13.56	51.83	8.32	8.84
11	2/3	0	1/3	12.03	10.25	11.05	11.70	5.46	4.48	8.65	8.64	21.56	51.41	8.71	13.24
12	1/6	2/3	1/6	10.71	9.268	19.72	12.12	5.05	5.09	8.68	8.33	43.37	52.05	9.48	17.82
13	0	2/3	1/3	11.91	12.10	14.94	13.70	4.81	4.62	8.40	7.51	24.85	21.43	10.92	18.88
14	1/3	1/3	1/3	12.40	13.91	9.13	14.18	4.71	5.20	8.26	8.05	46.53	48.59	10.25	18.82

[a] TPC-L: total phenolic content for leaves, TPC-I: total phenolic content for inflorescences is expressed in mg GAE per g of dry powder, total antioxidant capacity (TAC), cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), DPPH and ABTS-radical scavenging are expressed in mg Trolox equivalent per g of dry powder (mg TE g of dry powder).

2.2 Plant material

The National Agency of Medicinal and Aromatic Plants (ANPMA, Taounate, Morocco) provided cannabis (*Cannabis sativa* L.) seeds of a local ecotype "Beldia". In February 2025, seeds were sown and grown in 300 pots under plastic tunnels conditions at daytime temperatures of 35–40 °C. During the growing period (March–June), leaves were harvested successively between March and April 2025 during the vegetative stage, whereas female inflorescences were collected during the flowering stage between May and June 2025 after sex identification based on inflorescence development. To obtain representative and homogeneous samples, plant material from several plants was pooled separately according to organ type (leaves and inflorescences). The collected material was dried in a ventilated oven at 40 °C, ground into a fine homogeneous powder with a particle size < 0.33 mm, and stored at 4 °C into closed plastic test tubes until analysis.

2.3 Optimization and experimental design

A simplex lattice mixture design was used to evaluate the effect of solvent mixtures on the antioxidant activity and phenolic content of cannabis leaves and inflorescences. Three solvents (water, ethanol, and acetone) were selected on the basis of preliminary tests performed with water, 2-propanol, ethanol, and acetone for phenolic compound extraction. Table 1 summarizes the fourteen extraction experiments carried out. Binary and ternary solvent combinations were evaluated using a three-component design with six levels and one repetition at the central point (a ternary mixture with equal proportions). The dependent response variables (Y_i) were total phenolic content (TPC), total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), DPPH radical-scavenging activity, and ABTS radical cation-scavenging activity.

The relationship between the responses and the independent variables was described using the polynomial regression models shown in eqn (1)–(4).

$$\text{Linear}: \quad y_i(x) = \sum_{i=1}^{q} \beta_i x_i \tag{1}$$

$$\text{Quadratic}: \quad y_i(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q} \beta_{ij} x_i x_j \tag{2}$$

$$\text{Special cubic}: \quad y_i(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum_{i<j<k}^{q} \beta_{ijk} x_i x_j x_k \tag{3}$$

$$\text{Full cubic}: \quad y_i(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum_{i<j}^{q} \delta_{ij} x_i x_j (x_i - x_j) + \sum_{i<j<k}^{q} \beta_{ijk} x_i x_j x_k \tag{4}$$

where β_i, β_{ij}, and β_{ijk} are the regression coefficients for each corresponding interaction term.

2.4 Extract preparation from cannabis inflorescences and leaves

Each extract was prepared by mixing 2 mL of solvents with 0.2 g of plant powder (solid-to-liquid ratio 1 : 10 w/v). The mixture was vortexed for 5 min followed by sonication in an ultrasound bath for 30 min (Transonic T460 Germany35 KHz) in a cold room. The tubes were centrifuged for 10 min at 8965 g and the supernatant was recovered after two successive extractions and preserved at 4 °C until analysis. A total of fourteen extracts were obtained for each plant matrix. All antioxidant tests and chemical essays were performed on the recovered supernatants directly without solvent evaporation or isolation of dried extracts. The analytical results were therefore expressed relative to the initial dry weight of the plant material as dry weight inflorescences (DWI) and dry weight leaves (DWL), allowing comparison among the different solvent mixtures under identical extraction conditions.

Several experiments focused on either scavenging free radicals or reducing capacity were used to assess the antioxidant properties of cannabis leaf and inflorescence extracts. A UV-visible spectrophotometer (Jenway 7305, France) was used to measure absorbance for all of these spectrophotometric tests. The findings were reported as mg Trolox equivalent per g of dry weight (mg TE per g DW) using a Trolox (tetramethylchroman-2-carboxylic acid) calibration curve (0.039–1.25 mg mL^{-1}).

2.4.1 DPPH scavenging activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a purple free radical that becomes yellow upon reduction by antioxidant compounds. Briefly, 1 mL of a methanolic DPPH solution (0.04 mg mL^{-1}) reacted with 50 μL of each extract in dark conditions for 30 min. The absorbance was then measured at λ = 517 nm.[7] Trolox was used as the standard ($y = -1.3805x + 0.824$; $R^2 = 0.990$).

2.4.2 ABTS radical cation scavenging assay. The ABTS radical cation scavenging assay was carried out according to Benkirane *et al.*[7] The $ABTS^{*+}$ was produced by oxidation of ABTS (2,2′- azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) with potassium persulfate. Briefly, a 7 mM methanolic solution of ABTS was mixed with aqueous solution of 2.45 mM of potassium persulfate and allowed to react for 12–16 hours in the dark to form the ABTS radical cation. The assay is based on a decolorization mechanism in which the blue–green color of $ABTS^{*+}$ is reduced in the presence of antioxidants. For this, 50 μL of each extract was added to 1 mL of the $ABTS^{*+}$ solution after adjusting the absorbance to 1.0 at $\lambda = 734$ nm. The solution was completely mixed and kept to react for 30 min at room temperature. Trolox was used as the standard ($y = -1.1353x + 1.0153$, $R^2 = 0.9993$).

2.4.3 Total antioxidant capacity. For the total TAC assay, the phosphomolybdenum reagent was prepared by mixing 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid in equal volumes.[10] Then, 1 mL of this reagent was mixed with 10 μL of the extract. The tubes were vortexed and incubated in a water bath at 95 °C for 90 min, and absorbance was measured at λ = 695 nm. A Trolox calibration curve ($y = 0.3991x - 0.0254$; $R^2 = 0.9908$) was used for

quantification, and the antioxidant activity was reported as mg Trolox equivalents per gram of dry weight (mg TE per g DW).

2.4.4 Ferric reducing antioxidant power (FRAP). The FRAP assay is based on reducing Fe^{3+} present in the $K_3Fe(CN)_6$ complex to Fe^{2+}, as described in the literature.[11] Briefly, 65 μL of phosphate buffer (0.2 M, pH 6.6) was added to 10 μL of extract and 65 μL of 1% ferricyanide. The mixture was placed in a water bath at 50 °C for 20 min. To stop the reaction, 65 μL of 10% trichloroacetic acid was added. Then, 65 μL was collected from each tube and mixed with 65 μL of water and 25 μL of 1% $FeCl_3$. The absorbance was measured at $\lambda = 700$ nm. Trolox was used as the standard ($y = 0.2996x + 0.3687$; $R^2 = 0.9922$).

2.4.5 Cupric reducing antioxidant capacity (CUPRAC). The CUPRAC assay is based on the reduction of Cu^{2+} to Cu^{+} by antioxidants, as described by Benkirane *et al.*[7] Briefly, 300 μL of the reagent mixture (10 mM $CuCl_2$, 1 M ammonium acetate, and 7.5 mM neocuproine, 1 : 1 : 1, v/v/v) was mixed with 10 μL of extract. After 30 min of incubation the absorbance was measured at $\lambda = 450$ nm. Trolox was used as the standard ($y = 0.8179x + 0.0242$; $R^2 = 0.986$).

2.5 Total phenolic content

The total phenolic content (TPC) was quantified using the Folin–Ciocalteu method as previously described by Mansouri *et al.*[12] with some modifications. 1 mL of the Folin–Ciocalteu reagent and 1 mL of 10% sodium carbonate were combined with 20 μL of extract. The mixture was incubated for 60 min in a dark room, and the absorbance was measured at $\lambda = 760$ nm. TPC was quantified using a calibration curve of gallic acid at different concentrations (0.017–0.5 mg mL^{-1}); the limits of detection (LOD) and quantification (LOQ) were 83.30 and 252.41 μg mL^{-1}, respectively. The gallic acid was used as a standard ($y = 0.7742x - 0.0144$; $R^2 = 0.9986$). Milligram gallic acid equivalents (GAE) per g of dry weight (DW) was used to express the results (mg GAE per g DW) for both matrices.

2.6 HPLC-DAD and MS analyses of phenolic compounds

An Agilent 1260 infinity II high-performance liquid chromatography system (HPLC Agilent Technologies, USA) coupled to a diode array detector (DAD) was used to separate phenolic compounds on an Eclipse XDB-C18 column (3.5 μm particle size, 150 × 4.6 mm, Agilent Technologies USA). Water and acetonitrile with 1% formic acid formed the mobile phase. The chromatographic gradient was adapted from Benkirane *et al.*[7] The extract (10 μL) was injected at a rate of 0.6 mL min^{-1}. The separation of sample was monitored at wavelengths of 254, 280, 300, and 340 nm, and UV-visible spectra were obtained between 190 and 800 nm. Following their separation, each peak was gathered in 2 mL vials at HPLC system's output and identified using mass spectrometry.

The collected peaks were directly infused (at 500 μL h^{-1}) into an ion-trap mass spectrometer (Esquire HCT mass spectrometer, Bruker Daltonics, Germany) equipped with an electrospray ionization (ESI) source in both positive and negative ionization modes. The following ESI operating parameters were established using smart mode with a target mass of 400, 500, and 600 *m*/*z*: spray voltage of 4500 V, dry gas temperature of 200 °C, nebulizer pressure of 10 psi, and dry gas flow rate of 4 L min^{-1}. After isolating the precursor ion inside the ion trap, mass MS^2 spectra were generated for every mass scan using an adjustable collision energy of 1–10%. MS and MS^2 spectra were obtained in ultra-scan mode using a mass range of 50–1000 *m*/*z* at a speed of 26 000 *m*/*z*/*s*. The ESI tuning mix was used to calibrate the device. ACDlabs 2025 was used for mass data processing while Esquire Control software was utilized for instrument control and data collecting.

The Open LAB software was used to evaluate and analyze the HPLC-DAD profiles. Chromatographic peak areas obtained at 280 nm were used for the semi-quantification of the identified compounds since most phenolic compounds exhibit strong absorption at this wavelength. Owing to the limited commercial availability of analytical standards for several identified compounds, semi-quantification was performed using representative external calibration curves. Cannabinoids were semi-quantified using a CBN calibration curve (3–100 μg mL^{-1}, LOD = 12.51 μg mL; LOQ = 37.92 μg mL^{-1}), and results were expressed as mg CBN equivalents per g dry weigh (mg CBNE per g DW). Hydroxycinnamic acid amides were semi-quantified using an *N-trans*-caffeoyltyramine calibration curve (60–980 μg mL^{-1}, LOD = 34 μg mL^{-1}, LOQ = 102 μg mL^{-1}) and expressed as mg *N-trans*-caffeoyltyramine equivalents per g dry weight (mg CTE per g DW). Phenolic acids were quantified using a ferulic acid calibration curve (15–1000 μg mL^{-1}, LOD = 27.60 μg mL^{-1}, LOQ = 83.66 μg mL^{-1}) and expressed as mg ferulic acid equivalents per g dry weight (mg FAE per g DW). Flavonoids were semi-quantified using a quercetin calibration curve (15–1000 μg mL^{-1}, LOD 36.33 μg mL^{-1}, LOQ = 110.09 μg mL^{-1}) and expressed as mg quercetin equivalents per g dry weight (mg QE per g DW). The use of class-specific reference standards (CBN, *N-trans*-caffeoyltyramine, ferulic acid, and quercetin) allowed semi-quantitative comparison within each compound class. However, because individual compounds may differ in their UV absorption responses from the corresponding reference standard, the reported values represent equivalent estimates and should not be interpreted as absolute concentrations of individual compounds. This consideration is particularly relevant for cannabinoids, for which acidic forms such as Δ9-THCA and CBDA may exhibit different UV absorption characteristics from their neutral counterparts, including CBN.

2.7 Statistical analysis

Statistica software (version 10.0 StatSoft Inc. USA) was used to conduct statistical analysis. Analysis of variance (ANOVA), coefficient of determination (R^2), adjusted R^2, and lack-of-fit tests were used to evaluate the regression models generated from the mixture design. Data were reported as mean ± standard deviation. Differences were considered statistically significant at $p < 0.05$. Pearson's correlation analysis was performed separately for leaves and inflorescences to assess the relationships between TPC and antioxidant responses (DPPH, ABTS, FRAP, CUPRAC, and TAC). Statistical significance was considered at $p < 0.05$.

3 Results and discussion

The mixture design approach was applied independently to leaves and inflorescences to generate mathematical models for each matrix linking solvent proportions to chemical and antioxidant responses for each plant matrix. The predictive models were then represented as contour diagrams that illustrate the evolution of responses as a function of the solvent composition. The experimental response values (TPC, DPPH, ABTS, FRAP, CUPRAC, and TAC) obtained for leaves and inflorescences are summarized in Table 1.

3.1 Total phenolic content

Bioactive compounds have gained increased attention for their various applications in the food, nutraceutical, pharmaceutical, and cosmetic industries.[13] *Cannabis sativa* inflorescences and leaves are potential sources of bioactive compounds, whose recovery is influenced by several parameters, particularly the solvent composition used.[6] In this study, TPC showed significant dependence on the solvent composition and plant organ (Table 1). In inflorescence extracts, the highest TPC value (16.056 mg GAE per g DWI) was obtained with an acetone-rich mixture (Run 5), whereas in leaf extracts the maximum TPC (13.65 mg GAE per g DWL) was obtained with an ethanol–acetone mixture (Run 9). A notable contrast was observed with pure water (Run 3), which yielded the lowest value for inflorescence (5.98 mg GAE per g DWI). In the same context, Aazza[6] highlighted that water extract gives the lowest TPC value (7.23 mg GAE per g dry powder) from *Cannabis sativa* waste. However, leaves showed different behavior using pure water (12.79 mg GAE per g DWL), which is consistent with other studies showing that polar solvents can recover high levels of polyphenols from cannabis leaves.[14] This difference between plant organs may be explained by differences in phenolic compound composition. Inflorescences may contain a higher level of non-polar compounds with limited water solubility, whereas leaves may be richer in hydrophilic phenolic compounds.

To describe the interaction between solvents and phenolic extraction, mixture design regression analysis was performed (SI Tables S1 & S2). TPC variability was best described by the special cubic model for leaf extracts ($p = 0.015$), whereas the quadratic model provided the best fit for inflorescence extracts ($p = 0.0053$). The high coefficient of determination R^2 and the non-significant lack of fit values confirmed the suitability of these models for explaining the experimental TPC data. In the fitted models, a positive regression coefficient indicates the variable's capacity to enhance the response, while a negative sign shows the opposite.[6] The quadratic model for TPC for inflorescence extracts indicated that the individual solvent coefficients of ethanol ($\beta_2 = 12.31$) and acetone ($\beta_3 = 16.50$) were more effective than pure water ($\beta_1 = 5.01$). Among the binary interactions the ethanol–acetone combination ($\beta_{23} = -5.35$) reflects an antagonistic effect, suggesting that combining these two solvents reduced extraction efficiency, possibly because of competitive solvent effects. In contrast, the synergistic interaction of water with both organic solvents (β_{12} and β_{13}) highlight the important role of water in swelling plant tissues and facilitating organic solvent penetration.[7]

For leaf extracts, the special cubic model for TPC included positive and significant coefficients for all pure solvents (β_1, β_2 and β_3). Additionally, the binary ethanol–acetone interaction coefficient ($\beta_{23} = 8.94$) demonstrated a synergistic effect, in contrast to the antagonistic interaction observed for inflorescence extracts. This difference suggests that the behavior of these two solvents depends on the plant matrix and highlights the variation of phenolic composition between leaves and inflorescences that may influence solvent performance. Moreover, the positive ternary interaction coefficient (β_{123}) observed for leaves indicates a complementary effect of water, ethanol,

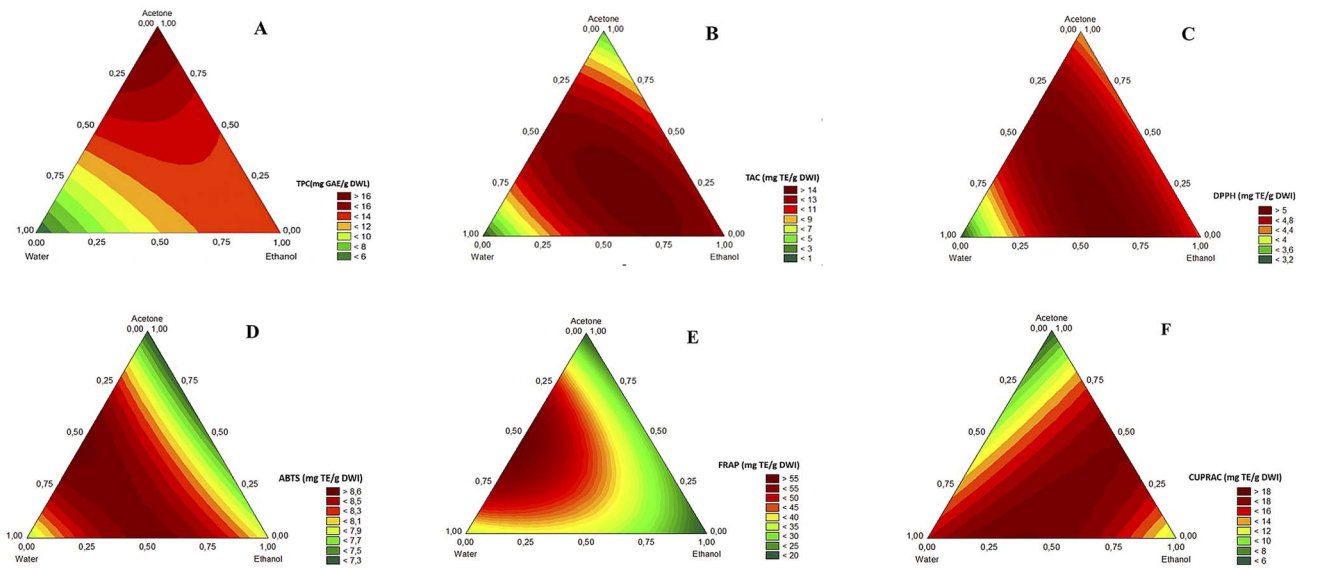

Fig. 1 Contour plots illustrating the effect of solvent composition on TPC (A), TAC (B), DPPH (C), ABTS (D), FRAP (E), and CUPRAC (F) responses of *Cannabis sativa* L. inflorescence extracts.

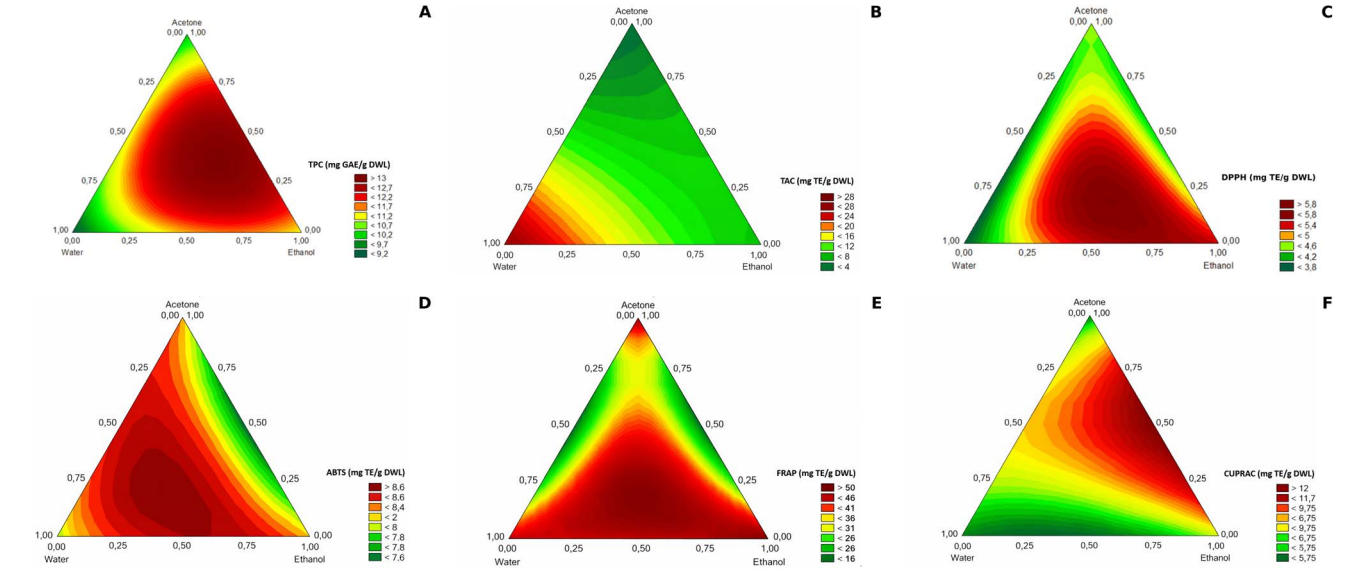

Fig. 2 Contour plots illustrating the effect of solvent composition on TPC (A), TAC (B), DPPH (C), ABTS (D), FRAP (E), and CUPRAC (F) responses of *Cannabis sativa* L. leaf extracts.

and acetone, likely resulting from their combined polarity range. In the same sense, many studies have also found a synergistic effect of water when combined with organic solvents, particularly acetone, ethanol, and methanol, on the extraction of phenols from different plant matrices.[7,15]

The contour plot for inflorescences in (Fig. 1A) was consistent with the fitted coefficients. The high TPC concentrations are observed in acetone rich regions, whereas pure water produced the lowest TPC. Additionally, we noticed that mixing water and acetone enhanced the ability to extract phenolic compounds. Compared with inflorescences, the contour plot for leaves (Fig. 2A) indicated a different solvent behavior. The highest TPC values were achieved near the ternary mixture region indicating that an effective extraction from leaves requires a combination of the three solvents. In contrast, acetone alone exhibited poor extraction efficiency for leaves.

3.2 Antioxidant activity

To evaluate the antioxidant capacity of cannabis extracts, five antioxidant assays (DPPH FRAP ABTS TAC and CUPRAC) were used to provide an overview of their radical-scavenging and reducing capacities. As reported by Chandimali *et al.*[19] antioxidants play a crucial role in limiting oxidative stress caused by free radicals, and their efficacy depends on the combination of several antioxidant mechanisms. Therefore, a single method is insufficient to determine the potential of plant extracts, which justifies the application of complementary antioxidant tests in this study. As shown in Table 1, the antioxidant activity for inflorescence extracts ranged from 3.53–15.70, 3.12–5.20, 7.16–8.68, 16.38–52.79, and 7.27–18.88 mg TE per g DWI for TAC, DPPH, ABTS, FRAP, and CUPRAC, respectively. Leaves showed different ranges: 6.77–31.73, 3.72–5.78, 7.27–8.85, 13.56–51.55, and 3.37–14.90 mg TE per g DWL, respectively. As observed TPC, antioxidant activities also differed ($p < 0.05$) according to plant organ and solvent composition.

The results showed that leaves exhibited higher TAC, reaching a high value of 31.73 mg TE per g DWL (Run 10: 1/3 water–2/3 acetone), which is significantly higher than that recorded for inflorescences (15.70 mg TE per g DWI; Run 1: 1/3 water–2/3 ethanol). This finding suggests that leaf tissues contain a diverse pool of antioxidant metabolites that are more efficiently solubilized in acetone-based mixtures. Regarding radical scavenging activities (DPPH and ABTS) both organs

Table 2 Quadratic model coefficients for *Cannabis sativa* inflorescence extract[a]

Responses (Y_i)	Coefficients						*p*-Values		
	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	Model	Lack of fit	R^2
TPC	5.013*	12.312*	16.502*	9.941	10.441	−5.357	0.0053	0.084	0.902
TAC	0.893	12.529*	3.522*	24.167*	43.959*	11.573	0.0018	0.105	0.904
DPPH	4.494*	3.214*	4.242*	4.671*	0.308	5.424*	0.00011	0.272	0.939
ABTS	7.893*	7.968*	7.267*	2.798*	0.555	4.456*	0.00093	0.892	0.895
FRAP	25.756*	35.629*	19.254*	22.952	16.252	120.50*	0.0078	0.740	0.817
CUPRAC	16.062*	10.752*	5.946*	21.627*	0.722	38.514*	0.0012	0.229	0.914

[a] $Y_i = \beta_1$ water + β_2 ethanol + β_3 acetone + β_{12} water × ethanol + β_{13} water × acetone + β_{23} ethanol × acetone. *Significant at $p < 0.05$.

demonstrated similar performances. For DPPH, the highest activity was recorded in Run 4 (5.78 mg TE per g DWL) for leaves and in Run 14 (5.20 mg TE per DWI) for inflorescences. For ABTS, leaves reached a maximum of 8.85 mg TE per g DWL (Run 8), while inflorescences reached 8.68 mg TE per g DWI (Run 2). Similar findings have been reported in showing that leaf extracts can exhibit greater antioxidant capacity than inflorescences in ABTS and DPPH radical scavenging tests. For instance, the DPPH antioxidant activity of cannabis leaves (20.73 mg TRX per g DM) was slightly higher than that of inflorescences (19.91 mg TRX per g DM), suggesting that leaf extracts may contain rapidly reacting antioxidants that contribute to higher scavenging values.[20]

Regarding CUPRAC assay, inflorescences reached 18.88 mg TE per g DWI (Run 13: 2/3 ethanol–1/3 acetone), exceeding the maximum value observed for leaves (14.90 mg TE per g DWL; Run 9). Similarly, the highest FRAP value (52.79 mg TE per g DWI) was recorded for inflorescences using the mixture of 1/6 water–1/6 ethanol–2/3 acetone (Run 5). This observation is consistent with a previous study showing that cannabis inflorescences have a considerably higher iron-reducing capacity, particularly in the FRAP assay (15.52 mg TRX per g dry matter) compared to leaves (8.67 mg TRX per g DM).[20] These findings suggest that the specific metabolites like cannabinoids that are concentrated in the floral trichomes are highly efficient in electron-transfer mechanisms. Furthermore, these results highlight the influence of plant organ on antioxidant activity.[20,21]

Mixture design regression was used to study the effect of solvent mixture on the antioxidant responses. For inflorescence extracts, the responses were fitted by a quadratic model ($p < 0.01$), with high R^2 values (0.817–0.939) and non-significant lack of fit values (Table 2). The regression coefficients indicated that pure ethanol (β_2) and water (β_1) contributed more strongly to FRAP and CUPRAC activities than pure acetone (β_3). Except for TAC, the ethanol–acetone combination (β_{23}) contributed positively to the extraction of antioxidants, indicating a synergistic effect for FRAP ($\beta_{23} = 120.50$) and CUPRAC ($\beta_{23} = 38.51$), conversely to the antagonistic effect observed for TPC. This synergistic effect is consistent with the high CUPRAC and FRAP values observed for ethanol–acetone mixtures (Table 1). These results are confirmed by several studies demonstrating that an intermediate polarity leads to higher phenolic compound content and consequently enhanced antioxidant capacity in different matrices. For instance, a 50% ethanol/water mixture has been shown to improve phenolic extraction in the aerial parts of cannabis.[22] In addition, Dudley *et al.* used water, acetone, and ethanol to extract phenolic and flavonoid compounds from female cannabis inflorescences. Their results showed that ethanolic extracts increased the antioxidant activity tested by DPPH (64.03%), together with a significantly higher extraction yield and bioactivity.[23]

For leaf extracts, the experimental data were best described by a special cubic model (Table 3). The linear coefficients were positive and significant for almost all pure solvents. However, many binary interaction coefficients (β_{12}, β_{13}, and β_{23}) were negative, particularly for FRAP and ABTS, indicating antagonistic effects. In contrast, the ternary interaction (β_{123}) showed positive coefficients for most responses, showing a synergistic effect when the three solvents were combined. These findings highlight that ternary mixtures are more suitable for extracting antioxidant phenolic from leaves than binary mixtures or pure solvents. A comparable result was reported by Alcântara *et al.*[24] who showed a positive ternary interaction between water–acetone–ethanol mixture in chia seeds. Such ternary systems provide a broad polarity range that enhances solvent penetration and extraction of diverse phenolic compounds.

The contour plots illustrate the dependence of antioxidant activities on solvent composition. The FRAP contour plot showed distinct behaviors between leaf and inflorescence extracts indicating a matrix-dependent effect on antioxidant extraction. For leaves, the highest reducing capacity was observed in the ternary solvent region (Fig. 2E), where the reducing power increased when water and organic solvents are incorporated. In contrast, inflorescence extracts exhibited a remarkable different pattern. The highest reducing power was observed in acetone–ethanol rich mixtures, while pure water showed low reducing capacity. Although the ethanol–acetone mixture extracted a lower total phenolic content, it produced enhanced antioxidant activity. This suggests that antioxidant performance is not governed solely by TPC but also by the chemical nature of the extracted compounds. The TAC profiles also showed distinct differences between the two organs: leaves (Fig. 2B) showed a high activity localized near the water–acetone mixture, whereas inflorescences (Fig. 1B) exhibited an optimal zone in central ternary region. The contour plots of DPPH (Fig. 1C & 2C) and ABTS (Fig. 1D & 2D) showed slightly higher activity and broader high-response zones for leaves than for

Table 3 Special cubic coefficients for *Cannabis sativa* model coefficients for leaf extract[a]

Responses (Y_i)	Coefficients						*p*-Values		
	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	Model	Lack of fit	R^2
TPC	9.266*	11.274*	9.726*	5.296	5.982	8.942*	12.244	0.015	0.577
TAC	28.640*	8.641*	3.884	−14.86*	−22.518	9.378	2.920	0.0001	0.003
DPPH	3.796*	5.396*	4.597*	3.362*	−0.89	−2.828	29.266*	0.002	0.532
ABTS	8.031*	8.201*	8.269*	2.344	1.550	−2.773*	10.245	0.04	0.101
FRAP	45.932*	52.262*	48.760*	−8.512	−125.91*	−140.38*	827.779*	0.002	0.100
CUPRAC	6.341*	3.508*	6.596*	−1.011	11.028*	32.319*	−3.751	0.0008	0.640

[a] $Y_i = \beta_1$ water + β_2 ethanol + β_3 acetone + β_{12} water × ethanol + β_{13} water × acetone + β_{23} ethanol × acetone. *Significant at $p < 0.05$.

Table 4 Tentatively identified compounds in *Cannabis sativa* L. leaf and inflorescence extracts

No peak	Compound name	Molecular formula	RT	$[M-H]^-$ (*m/z*)	MS^2 fragment	$[M+H]^+$ (*m/z*)	MS^2 fragment	Theoretical mass	UV λ_{max} (nm)
1	Coumaroyl gluconic acid	$C_{15}H_{18}O_9$	11.38	341.057	119(22),129(7.5), 135(5.5), 163(100) 164(4), 177(25), 179(25), 195(99), 295(3.5), 323(16)	—	—	342.095	194, 282
2	Coumaroyl heptosyl isomer-1	$C_{16}H_{20}O_9$	13.37	355.053	119(12), 129(4.5), 163(100), 191(5.5), 193(3), 209(55), 210(2), 212(2), 353(4)	—	—	356.110	194, 312
3	Coumaroyl heptosyl isomer-2	$C_{16}H_{20}O_9$	17.95	355.046	119(10.5), 163(100), 164(6.5), 177(3), 178(2.5), 179(3), 191(4.5), 193(23.5), 195(6), 209(39)	—	—	356.110	194, 312
4	Cannabisin A	$C_{34}H_{30}N_2O_8$	19.71	593.072	327(100), 357(46), 473(40), 429 (21), 309(12), 447(10), 459(7), 594(11)	595.232	449(100), 431(44), 595(37), 383(21), 413(20),329(15), 353(10), 395(10), 299(7)	594.600	214, 272, 330
5	Vitexin-2″-*O*-rhamnoside	$C_{27}H_{30}O_{14}$	22.77	—	—	579.275	283(20)313(20), 337 (17),367(27), 379(14),397 (27),414(9), 415(56) 433(100)	578.163	232, 270, 328
6	Chrysoeriol-8-*C*-glucoside	$C_{22}H_{22}O_{11}$	24.66	—	—	463.217	313(6.5), 343(15), 367(7), 397(17.5), 409(7), 427(17.5), 445 (49), 463(100)	462.400	210, 254, 348
7	Sesqui CBGA	$C_{27}H_{40}O_4$	27.26	427.085	123(10.5), 205(2.5), 221(17), 249(50), 255(5), 267(2), 271(4), 383(100), 384(5), 409(2)	—	—	428.610	214, 268
8	Apigenin-7-*O*-glucuronide	$C_{21}H_{18}O_{11}$	28.88	445.034	175(19), 269 (100), 270 (15), 325(1), 381(7.5), 399(1), 433(14), 445(4), 446(4)	447.164	271(100), 272(13), 448(4)	446.085	204, 266, 336
9	*N-trans*-Feruloyltyramine	$C_{18}H_{19}NO_4$	29.24	—	—	314.251	117(3), 145(23), 177(100), 178(8), 191(2.5), 233(5), 281(15), 282(2), 313(19)	313.300	274
10	*N-trans*-Coumaroyltyramine	$C_{17}H_{17}NO_3$	29.53	—	—	284.174	91(1.5), 119(3.5), 147(100), 284(25), 795(35)	283.120	196, 222, 292
11	*N-cis*-Feruloyltyramine	$C_{18}H_{19}NO_4$	30.02	312.055	135(78), 148(18), 176(12.5), 177(13), 178(100), 179(7), 297(85), 298(8), 311(16), 312(36)	314.189	117(2.5), 145(10.5), 146(1), 177(100), 178(8.5), 297(2), 314(3)	313.300	196, 220, 286, 314
12	Cannflavin B	$C_{21}H_{20}O_6$	30.73	367.069	148(24), 163(100), 164(4.5), 203(1.5), 247(2), 339(1.5), 349(1.5), 350(1), 366(10), 367(2.5)	369.680	105(47), 119(18), 233(6), 249(20), 324(8), 341(5.5), 351(12), 352(8), 353(7.5), 368(100)	368.400	218, 272, 340
13	Acacetin 7-*O*-glucuronide	$C_{22}H_{20}O_{11}$	31.06	459.075	173(4), 175(59), 283(100), 413(5.5), 438(7.5), 439(7), 456(66), 457(13), 459(11.5)	461.151	285(100), 286(11), 462(14)	460.100	210, 268, 332
14	CBTA-isomer-1	$C_{22}H_{30}O_6$	38.73	389.167	343(7), 344(11), 345(5), 353(15), 354(2.5), 369(2), 370(10), 371(100), 372(21), 373(2.5)	—	—	390.206	200, 222
15	CBEA	$C_{22}H_{30}O_5$	38.93	373.18	297(3), 299(5), 311(3.5), 329(100), 330(17), 355(70), 356(15), 357(3), 372(5), 373(13.5)	375.000	237(5), 313(26), 314(6), 327(5), 337(10), 339(7.5), 355(100), 357(71), 373(7.5)	374.209	220, 278, 302
16	CBTA-isomer-2	$C_{22}H_{30}O6$	39.20	389.170		—	—	390.206	222, 300

Table 4 (*Contd.*)

No peak	Compound name	Molecular formula	RT	$[M-H]^-$ (*m*/*z*)	MS^2 fragment	$[M+H]^+$ (*m*/*z*)	MS^2 fragment	Theoretical mass	UV λ_{max} (nm)
					236(10), 246(1), 264(21), 285(6), 309(6), 327(7.5), 345(5), 353(7.5), 371(100), 389(4)				
17	CBTA-isomer 3	$C_{22}H_{30}O_6$	39.84	389.132	205(2.5), 285(2.5), 301(2.5), 309(5), 327(6.5), 353(29), 354(7.5), 371(100), 372(32.5), 373(8)	—	—	390.206	222, 270, 318
18	D9-THCMA isomer-1	$C_{23}H_{32}O_4$	40.51	371.178	191(11.5), 192(1.5), 309(3), 310(1), 327(26), 328(3.5), 353(100), 354(18), 355(4), 371(4)	—	—	372.221	224, 268, 306
19	CBNDA	$C_{22}H_{26}O_4$	40.83	353.164	267(1.5), 309(100), 310(12.5), 353(1.5)	355.234	213(3), 267(1), 283(2), 295(3), 297(2.5), 309(3.5), 319(2.5), 327(6.5), 337(100), 338(12)	354.190	220, 266, 308
20	D9-THCMA-isomer-2	$C_{23}H_{32}O_4$	41.75	371.126	309(5), 310(1), 325(2), 326(1.5), 327(71), 328(15), 353(100), 354(20), 369(14), 371(1.5)	373.233	235(1), 315(27), 316(5), 354(6), 355(100), 356(31), 357(3), 373(1.5)	372.221	228, 270, 306
21	CBGMA-isomer-1	$C_{23}H_{34}O_4$	42.33	373.145	173(6), 191(7), 268(5), 301(4), 311(100),312(9.5), 329(9.5), 354(25), 355(97), 356(9)	—	—	374.237	219, 260
22	CBGMA-isomer-2	$C_{23}H_{34}O_4$	42.96	373.145	173(8.5), 205(56.5), 259(9),271(34), 311(74), 329(100), 330(9), 355(12.5), 371(17.5)	—	—	374.237	222, 272
23	CBDA	$C_{22}H_{30}O_4$	43.39	357.157	179(2.5), 271(1), 289(1), 311(9.5), 312(1), 313(4), 339(100), 340(8.5)	359.248	341(100), 342(14), 359(13), 360(15), 361(3.5)	358.206	240, 274, 312
24	CBD	$C_{21}H_{30}O_2$	43.96	313.146	135(2), 137(1.5), 179(37.5), 180(3), 245(55), 246(7.5), 297(1), 311(100), 312(15), 313(14)	315.297	135(39), 181(27), 193(77), 207(20), 221(24), 231(17), 233(40), 235(35), 259(100), 260(20), 300(14), 315(7.5)	314.216	210, 274
25	Δ9-THCVA	$C_{20}H_{26}O_4$	45.95	329.138	151(2.5), 163(2), 217(5), 283(4.5), 285(100), 311(7), 329(15)	—	—	330.190	222
26	CBDVA	$C_{20}H_{26}O_4$	46.51	329.127	151(5), 163(5.5), 202(1.5), 267(1.5), 283(16), 285(17.5), 311(100), 312(12), 329(2.5)	—	—	330.190	222
27	CBCVA	$C_{20}H_{26}O_4$	47.40	329.171	151(5.5), 163(8.5), 201(2.5), 233(7), 248(3.5), 283(23.5), 285(28), 286(3), 311(100), 312(11.5)	—	—	330.190	222
28	CBNA	$C_{22}H_{26}O_4$	47.68	353.153	265(1), 267(2.5), 309(100), 310(13)	—	—	354.190	220, 266
29	Δ9-THCA-isomer-1	$C_{22}H_{30}O_4$	48.59	357.138	179(2), 191(1.5), 245(5), 311(6.5), 313(100), 314(10), 339(7.5), 356(2)	—	—	358.206	226, 272, 306
30	Δ9-THCA-isomer-2	$C_{22}H_{30}O_4$	49.06	357.202	179(1), 191(2), 245(2.5), 311(7), 313(100), 314(8.5), 315(1), 339(15), 340(1), 357(4)	—	—	358.206	226, 272, 306, 434
31	CBCA	$C_{22}H_{30}O_4$	49.76	357.129	179(5), 191(4.5), 274(2), 311(12), 313(20), 314(2.5), 339(100), 340(10), 357(3)	—	—	358.206	254, 294, 328
32	CBLA	$C_{22}H_{30}O_4$	50.00	357.136	179(8), 191(7.5), 261(13), 276(3), 311(19), 313(45), 314(3.5), 339(100), 340(10)	—	—	358.206	224, 434, 412

inflorescences. In contrast, the CUPRAC plots (Fig. 1F & 2F) showed higher reducing capacity for inflorescences. In fact, displayed moderate activity mainly in the acetone–ethanol region, whereas inflorescences showed an extensive high-response region across a wide range of solvent compositions particularly ethanol-rich mixtures. These findings suggest that organic solvent synergies, especially ethanol–acetone mixtures, enhance reducing capacity in both matrices.

Pearson's correlation analyses between TPC and DPPH revealed a significant positive correlation in both leaves ($r = 0.606$; $p = 0.022$) and inflorescences ($r = 0.569$; $p = 0.034$). In contrast, no significant correlations were observed between TPC and ABTS, FRAP, or CUPRAC ($p > 0.05$). In leaves, a negative correlation between TPC and TAC ($r = -0.790$; $p = 0.001$), whereas no significant correlation was found in inflorescences (SI Table S3). Similar correlations between phenolic content and antioxidant activity, especially DPPH, have reported in previous studies. However, the strength of these correlations varies depending on the antioxidant assay employed and extraction conditions.[16–18] These variations suggest that antioxidant activity is influenced not only by the total phenolic content but also on the phytochemical composition of the extracts and the underlying mechanisms involved in each assay.

3.3 Phenolic profile of leaf and inflorescence extracts

The phenolic profile of each extract was examined to evaluate the influence of solvent composition on compound recovery. After HPLC-DAD separation (SI Fig. S1), ESI-MS/MS analysis was used to further characterize the detected peaks. Precursor ions and MS^2 fragmentation patterns obtained in positive and negative ionization modes (SI Fig. S2–S4) were compared with data published in the literature.[25–28] A total of 32 phenolic compounds were tentatively identified in both matrices (leaf and inflorescence extracts), comprising phenolic acids, hydroxycinnamic acid amides, flavonoids, lignanamides, and cannabinoids. Table 4 summarizes the annotated compounds, with experimental and theoretical m/z, MS^2 fragments, molecular formula, retention times, and UV λ_{max} values.

The results showed that cannabinoids represent a major subclass among the identified compounds in both matrices. In total, 20 different cannabinoids were tentatively identified. Several major acidic cannabinoids shared the same molecular formula and exhibited similar deprotonated molecular ions at approximately m/z 357 in negative ionization mode, as previously reported for cannabidiolic acid (CBDA) and Δ9-tetrahydrocannabinolic acid (Δ9-THCA).[3] These compounds could not be distinguished based only on their exact molecular mass, but they were differentiated using their elution order and diagnostic fragment ion intensities (Fig. 3).

In negative ionization mode, CBDA and Δ9-THCA produced similar fragment ions (m/z 313 and m/z 339) (Fig. 4). However, the relative intensities of these diagnostic ions were different. For compound **23**, the dominant fragment in the MS^2 spectrum was the result of a dehydration (−18 Da), indicating a loss of H_2O while decarboxylation fragment (m/z 313) was less intense. This fragmentation pattern associated with CBDA is consistent with the open ring structure of CBDA. On the other hand, compounds **29** and **30** showed an intense fragment ion at m/z 313, resulting from the loss of CO_2 (−44 Da), which supports the presence of a carboxylate group. Thus, retention time and MS^2 fragmentation provided sufficient evidence to distinguish CBDA from Δ9-THCA despite their identical nominal mass. Compound **29** exhibited the same deprotonated molecular ion ($[M - H]^-$ at m/z 357) and molecular formula ($C_{22}H_{30}O_4$) as compound **30**, with similar MS^2 fragment ions but a distinct retention time. Accordingly, Δ9-THCA was tentatively assigned to compound **29** at 49.06 min, while compound **30** at 48.63 min was assigned to a structural isomer.

Compound 7 was detected at 27.26 min with a deprotonated molecular ion mass $[M - H]^-$ at m/z 427.085. Its MS^2 spectrum showed a dominant fragment at m/z 383, corresponding to neutral loss of CO_2 (44 Da), indicating the presence of a carboxylic acid function and function and supporting its assignment as an acidic cannabinoid. Additional fragment ions

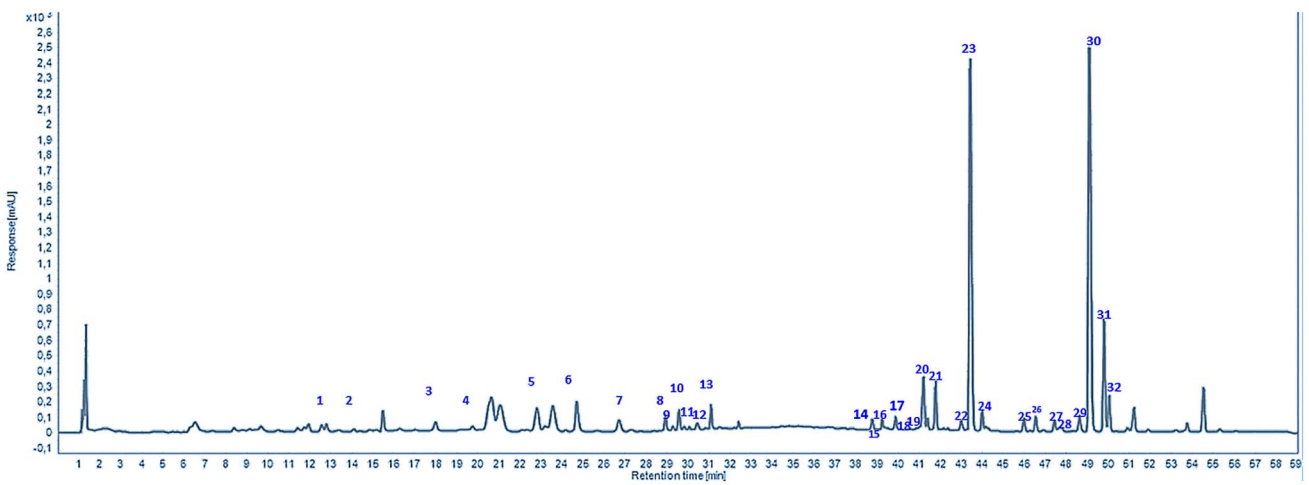

Fig. 3 HPLC chromatogram recorded at 280 nm for the extracted compounds from *Cannabis sativa* L. inflorescence.

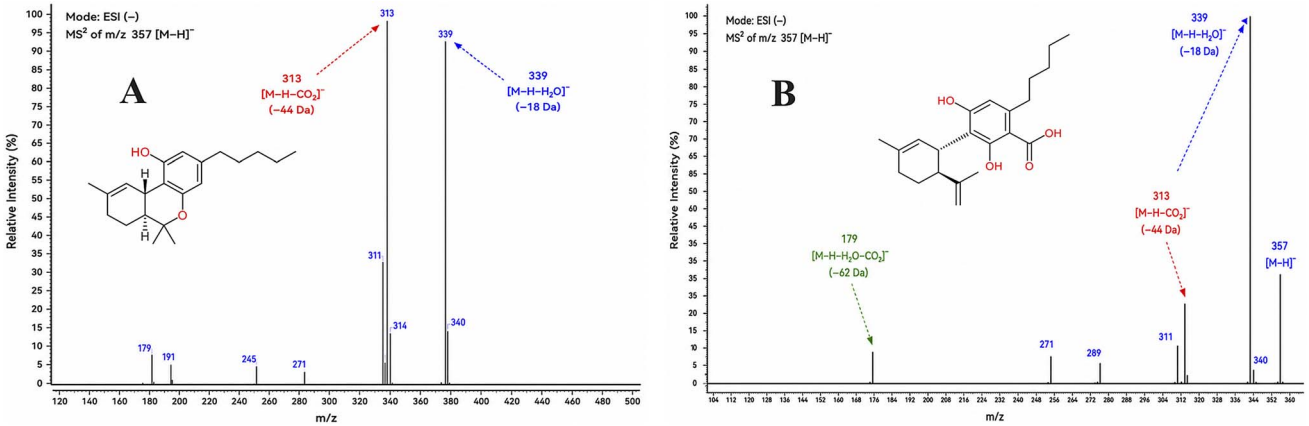

Fig. 4 MS^2 spectrum of (A) Δ9-THCA isomer 2 and (B) CBDA acquired in negative ionization mode (ESI^-).

were consistent with previously reported fragmentation patterns of sesquicannabigerolic acid (Sesqui-CBGA) in *Cannabis sativa*.[26,29] Therefore, based on *m*/*z* data and MS^2 fragmentation, compound **7** tentatively assigned as Sesqui-CBGA.

Two additional peaks detected at 49.77 and 50.00 (compounds **31** and **32**) min were tentatively assigned as cannabichromenic acid (CBCA) and cannabicyclolic acid (CBLA), respectively. Compound **31** eluted after Δ9-THCA under the same chromatographic conditions, indicating differences in polarity.[30] The compound that was assigned as CBLA exhibited the longest retention time, suggesting high hydrophobic interactions with the stationary phase. Moreover, both compounds showed the same molecular ion at *m*/*z* 357 and an intense MS^2 fragment at *m*/*z* 339, corresponding to possible loss of H_2O (−18 Da), which is typical for acidic cannabinoids. Based on MS^2 fragmentation pattern, UV λ_{max}, and elution order, they were tentatively identified as CBCA and CBLA. Compounds **14**, **16** and **17** showed a precursor ion $[M - H]^-$ at *m*/*z* 389 and a dominant fragment at *m*/*z* 371, corresponding to the loss of H_2O (−18 Da), together with fragments at *m*/*z* 353, 345, and 327. This fragmentation pattern, involving successive losses of H_2O and CO_2, is compatible with hydroxylated acidic cannabinoids. Based on fragmentation patterns and in comparison with literature data, the three compounds were tentatively assigned to cannabitriolic acid (CBTA) isomers, which are hydroxylated derivatives of Δ9-THCA bearing two additional hydroxyl groups at different positions.[26]

In addition to the acidic cannabinoids, CBD was also identified in negative and positive ionization mode (compound **24**), $[M - H]^-$ at *m*/*z* 313 and $[M + H]^+$ at *m*/*z* 315, respectively. In positive mode, the product ion at *m*/*z* 259 (base peak) likely resulted from loss of a C_4H_8 group from the alkyl side alkyl chain, whereas in negative mode the fragmentation of CBD produced an ion at *m*/*z* 245 due to loss of C_5H_8.[31,32] Additionally, the presence of CBD may be attributed to the partial decarboxylation of CBDA, specially it's expected to be low in inflorescence and leaf matrices of *Cannabis sativa* L.[25]

Several cannabinoid derivatives were also tentatively identified in this study, including cannabidivarinic acid (CBDVA), cannabichromevarinic acid (CBCVA), cannabinolic acid (CBNA), cannabinodiolic acid (CBNDA), which are fully aromatized forms of CBDA, as well as Δ9-tetrahydrocannabivarinic acid (Δ9-THCVA) and methylated homologues, such as cannabigerolic acid monomethyl ether (CBGMA) and Δ9-tetrahydrocannabinolic acid monomethyl ether (Δ9-THCMA). These compounds were less abundant than the predominant acidic cannabinoids.[26,33]

In addition to cannabinoids, the phytochemical profile revealed the presence hydroxycinnamic acid amides (HCAA), which are formed by condensation of hydroxycinnamic acids with aliphatic or aromatic amines.[34] Compound **10**, detected in positive ion mode with $[M + H]^+$ at 284.174, generated a main fragment at *m*/*z* 147 (base peak), assignable to the loss of the tyramine moiety, which supports its identification as *N-trans*-coumaroyltyramine.[35] Compounds **9** ($[M + H]^+ = 314.251$) and **11** ($[M + H]^+ = 314.189$) were assigned as *N-trans*-feruloyltyramine and *N-cis*-feruloyltyramine, respectively. In positive mode, they produced a dominant ion at *m*/*z* 177 due to loss of the tyramine moiety, whereas in negative mode the *m*/*z* 148 fragment was attributed to loss of the feruloyl moiety after the CO Cα′ cleavage.[28,35,36] In addition, apigenin-7-*O*-glucuronide acacetin-7-*O*-glucuronide vitexin-2″-*O*-rhamnoside, and chrysoeriol-8-*C*-glucoside, previously reported as major flavonoids in *Cannabis sativa*,[28] were also identified in inflorescences and leaf extracts.

The concentration of identified compounds in inflorescence and leaf extracts showed a strong dependence on the solvent composition (Tables 5 and 6). Some compounds were detected only in specific solvent systems, whereas others were present across several extracts but at markedly different concentrations. In inflorescence extracts, solvent mixture had a significant effect on cannabinoid recovery. For both matrices, Δ9-THCA and CBDA were the predominant cannabinoids.

Run 13 (2/3 ethanol–1/3 acetone) recorded the highest inflorescence extraction efficiency, with 4.693 and 3.843 mg CBNE per g DWI for Δ9-THCA and CBDA, respectively. The same

Table 5 Semi-quantitative concentrations of identified compounds in *Cannabis sativa* L. inflorescence extracts

Compounds	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11	Run 12	Run 13
Phenolic acids													
Coumaroyl gluconic acid	0.041 ± 0.005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coumaroyl heptosyl-isomer-1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coumaroyl heptosyl-isomer-2	ND	ND	ND	ND	0.021 ± 0.013	ND	ND	0.119 ± 0.002	ND	0.013 ± 0.002	ND	ND	ND
Total phenolic acids	**0.041**	**ND**	**ND**	**ND**	**0.021**	**ND**	**ND**	**0.119**	**ND**	**0.013**	ND	**ND**	**ND**
Hydroxycinnamic acid amides (HCAA)													
N-trans-Feruloyltyramine	0.500 ± 0.024	0.573 ± 0.197	0.351 ± 0.019	0.655 ± 0.027	0.485 ± 0.006	0.241 ± 0.005	ND	0.542 ± 0.032	0.309 ± 0.010	0.531 ± 0.051	0.674 ± 0.045	0.345 ± 0.008	0.261 ± 0.001
N-trans-Coumaroyltyramine	1.793 ± 0.013	0.140 ± 0.034	1.227 ± 0.027	1.778 ± 0.086	1.749 ± 0.079	0.490 ± 0.010	0.295 ± 0.032	1.799 ± 0.092	0.640 ± 0.099	1.816 ± 0.134	1.788 ± 0.144	1.409 ± 0.095	0.560 ± 0.001
N-cis-Feruloyltyramine	0.385 ± 0.058	0.305 ± 0.027	0.225 ± 0.018	0.307 ± 0.012	0.396 ± 0.005	0.195 ± 0.00	ND	0.409 ± 0.001	0.273 ± 0.001	0.437 ± 0.009	0.571 ± 0.003	ND	0.259 ± 0.010
Total HCAA	**2678**	**1018**	**1803**	**2740**	**2630**	**0,926**	**0.295**	**2750**	**1222**	**2784**	**3033**	**1754**	**1.080**
Flavonoid													
Acacetin 7-*o*-glucuronide	0.722 ± 0.005	0.271 ± 0.001	0.293 ± 0.015	0.289 ± 0.002	0.862 ± 0.016	0.627 ± 0.001	0.322 ± 0.002	0.785 ± 0.018	0.695 ± 0.023	0.811 ± 0.001	0.327 ± 0.00	0.715 ± 0.063	0.669 ± 0.030
Apigenin-7-*o*-glucuronide	0.648 ± 0.017	0.700 ± 0.071	0.577 ± 0.053	0.803 ± 0.038	0.589 ± 0.034	0.295 ± 0.001	ND	0.738 ± 0.026	ND	0.706 ± 0.046	0.824 ± 0.008	0.483 ± 0.001	0.317 ± 0.005
Chrysoeriol-8-*c*-glucoside	1.675 ± 0.024	1.286 ± 0.055	0.686 ± 0.064	1.539 ± 0.046	1.208 ± 0.048	ND	ND	1.649 ± 0.007	ND	1.581 ± 0.064	1.693 ± 0.016	0.872 ± 0.009	ND
Vitexin-2″-*o*-rhamnoside	1.811 ± 0.019	1.610 ± 0.120	1.032 ± 0.014	1.728 ± 0.092	1.930 ± 0.018	0.537 ± 0.015	ND	1.765 ± 0.077	ND	1.488 ± 0.473	1.707 ± 0.078	1.630 ± 0.023	ND
Cannflavin B	0.441 ± 0.058	0.383 ± 0.025	0.310 ± 0.009	0.283 ± 0.002	0.267 ± 0.003	0.359 ± 0.004	0.635 ± 0.033	0.270 ± 0.005	0.397 ± 0.006	0.277 ± 0.005	0.287 ± 0.005	0.509 ± 0.022	0.366 ± 0.007
Total flavonoïdes	**5.297**	**4.250**	**2.898**	**4.642**	**4.856**	**1.818**	**0.957**	**5.207**	**1.092**	**4.863**	**4.838**	**4.209**	**1.352**
Lignanamides													
Cannabisin A	0.529 ± 0.020	0.452 ± 0.016	0.326 ± 0.084	0.427 ± 0.054	0.547 ± 0.004	ND	ND	0.763 ± 0.069	ND	0.654 ± 0.082	0.646 ± 0.145	0.429 ± 0.065	ND
Total lignanamides	**0.529**	**0.452**	**0.326**	**0.427**	**0.547**	**ND**	**ND**	**0.763**	**ND**	**0.654**	**0.646**	**0.429**	**ND**
Cannabinoids													
Sesqui CBGA	0.051 ± 0.004	0.042 ± 0.003	0.041 ± 0.005	0.058 ± 0.010	0.030 ± 0.002	ND	ND	ND	ND	0.043 ± 0.00	0.074 ± 0.001	0.047 ± 0.009	ND
CBTA isomer 1	0.075 ± 0.001	0.023 ± 0.00	ND	0.014 ± 0.00	0.068 ± 0.004	0.0027 ± 0.00	0.065 ± 0.003	0.067 ± 0.004	0.072 ± 0.007	0.060 ± 0.001	0.022 ± 0.00	0.055 ± 0.00	0.067 ± 0.005
CBEA	0.004 ± 0.001	ND	ND	ND	0.013 ± 0.003	0.011 ± 0.00	ND	0.013 ± 0.002	ND	ND	ND	0.007 ± 0.001	0.005 ± 0.001
CBTA isomer 2	0.061 ± 0.003	0.033 ± 0.00	0.005 ± 0.001	0.044 ± 0.00	0.067 ± 0.013	0.065 ± 0.008	0.058 ± 0.004	0.062 ± 0.008	0.062 ± 0.002	0.067 ± 0.002	0.053 ± 0.018	0.052 ± 0.002	0.062 ± 0.002
CBTA isomer 3		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 5 (*Contd.*)

Compounds	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11	Run 12	Run 13
	0.074 ± 0.018												
Δ9-THCMA isomer 1	0.007 ± 0.00	ND	ND	ND	0.0013 ± 0.00	ND	ND	ND	ND	ND	ND	ND	ND
CBNDA	0.013 ± 0.001	0.015 ± 0.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Δ9-THCMA isomer 2	0.338 ± 0.029	0.185 ± 0.020	ND	0.053 ± 0.007	0.193 ± 0.024	0.030 ± 0.00	0.016 ± 0.001	0.317 ± 0.094	0.016 ± 0.001	0.218 ± 0.005	0.308 ± 0.019	ND	0.035 ± 0.003
CBGMA isomer 1	0.018 ± 0.00	0.007 ± 0.00	ND	0.013 ± 0.00	0.021 ± 0.002	0.008 ± 0.001	ND	0.024 ± 0.001	0.024 ± 0.003	0.022 ± 0.001	ND	0.021 ± 0.00	ND
CBGMA isomer 2	0.073 ± 0.035	ND	ND	ND	0.019 ± 0.001	0.013 ± 0.001	0.017 ± 0.002	ND	0.014 ± 0.001	0.029 ± 0.00	0.013 ± 0.001	0.008 ± 0.001	0.010 ± 0.00
CBDA	3.536 ± 0.109	1.653 ± 0.011	0.142 ± 0.002	1.600 ± 0.026	3.840 ± 0.028	3.627 ± 0.038	3.622 ± 0.005	3.470 ± 0.001	3.811 ± 0.100	3.439 ± 0.272	1.924 ± 0.128	3.095 ± 0.328	3.843 ± 0.080
CBD	0.160 ± 0.004	0.055 ± 0.00	ND	0.056 ± 0.014	0.188 ± 0.001	0.158 ± 0.014	0.189 ± 0.002	0.158 ± 0.003	0.189 ± 0.008	0.165 ± 0.010	0.065 ± 0.008	0.147 ± 0.008	0.205 ± 0.009
Δ9-THCVA	0.109 ± 0.006	0.049 ± 0.004	0.0039 ± 0.00	0.051 ± 0.010	0.140 ± 0.002	0.136 ± 0.007	0.142 ± 0.009	0.115 ± 0.00	0.155 ± 0.001	0.120 ± 0.007	0.068 ± 0.014	0.108 ± 0.002	0.161 ± 0.029
CBDVA	0.129 ± 0.00	0.044 ± 0.00	ND	0.056 ± 0.021	0.069 ± 0.002	ND	ND	0.125 ± 0.014	ND	0.291 ± 0.039	0.107 ± 0.012	0.065 ± 0.012	ND
CBCVA	0.108 ± 0.005	0.015 ± 0.002	ND	ND	0.124 ± 0.010	0.111 ± 0.006	0.112 ± 0.006	0.080 ± 0.012	0.129 ± 0.003	0.084 ± 0.00	ND	0.099 ± 0.007	0.125 ± 0.011
CBNA	0.080 ± 0.001	0.051 ± 0.004	ND	0.044 ± 0.010	0.045 ± 0.013	0.085 ± 0.004	0.031 ± 0.001	0.026 ± 0.002	0.096 ± 0.014	0.053 ± 0.002	0.045 ± 0.011	0.079 ± 0.007	0.066 ± 0.013
Δ9-THCA isomer 1	0.145 ± 0.012	ND	ND	ND	0.165 ± 0.00	0.188 ± 0.017	0.164 ± 0.013	0.129 ± 0.012	0.197 ± 0.00	0.139 ± 0.010	ND	0.137 ± 0.010	0.196 ± 0.012
Δ9-THCA isomer 2	3.994 ± 0.121	1.737 ± 0.084	0.066 ± 0.014	1.589 ± 0.007	4.419 ± 0.004	4.301 ± 0.016	4.416 ± 0.002	4.123 ± 0.102	4.578 ± 0.112	4.027 ± 0.263	1.967 ± 0.212	3.974 ± 0.178	4.693 ± 0.182
CBCA	0.845 ± 0.028	0.260 ± 0.023	0.011 ± 0.00	0.159 ± 0.068	0.938 ± 0.006	0.858 ± 0.006	0.866 ± 0.002	0.824 ± 0.038	0.963 ± 0.036	0.770 ± 0.078	0.291 ± 0.010	0.735 ± 0.072	1.008 ± 0.080
CBLA	0.290 ± 0.006	ND	ND	ND	0.214 ± 0.001	0.030 ± 0.001	0.015 ± 0.00	0.288 ± 0.024	0.040 ± 0.001	0.650 ± 0.166	0.083 ± 0.004	0.174 ± 0.007	0.043 ± 0.012
Total cannabinoids	**10.113**	**4.177**	**0.272**	**3.743**	**10.564**	**9.630**	**9.718**	**9.894**	**10.353**	**10.183**	**5.026**	**8.811**	**10.527**

Table 6 Semi-quantitative concentrations of identified compounds in *Cannabis sativa* L. leaves extracts

Compounds	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11	Run 12	Run 13
Phenolic acids													
Coumaroyl gluconic acid	0.055 ± 0.009	ND	ND	ND	ND	ND	ND	ND	ND	0.022 ± 0.010	ND	ND	ND
Coumaroyl heptosyl-isomer-1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coumaroyl heptosyl-isomer-2	0.176 ± 0.019	0.074 ± 0.005	ND	0.067 ± 0.002	0.067 ± 0.010	ND	ND	0.114 ± 0.024	ND	0.097 ± 0.028	0.105 ± 0.014	ND	ND
Total phenolic acids	**0.232**	**0.074**	**ND**	**0.067**	**0.067**	**ND**	**ND**	**0.114**	**ND**	**0.097**	**0.105**	**ND**	**ND**
Hydroxycinnamic acid amides (HCAA)													
N-trans-Feruloyltyramine	0.853 ± 0.141	0.669 ± 0.001	ND	0.777 ± 0.045	0.896 ± 0.224	ND	ND	0.894 ± 0.103	0.178 ± 0.005	0.711 ± 0.031	0.524 ± 0.069	0.562 ± 0.174	0.233 ± 0.011
N-trans-Coumaroyltyramine	3.556 ± 0.093	2.843 ± 0.011	0.932 ± 0.105	2.352 ± 0.011	2.528 ± 0.012	0.623 ± 0.021	ND	3.083 ± 0.107	0.330 ± 0.009	2.798 ± 0.011	2.554 ± 0.225	1.800 ± 0.053	0.698 ± 0.184
N-cis-Feruloyltyramine	0.775 ± 0.048	0.669 ± 0.054	0.125 ± 0.001	0.850 ± 0.157	0.663 ± 0.121	0.279 ± 0.005	ND	0.904 ± 0.134	0.159 ± 0.013	0.661 ± 0.011	0.541 ± 0.032	0.535 ± 0.060	0.251 ± 0.005
Total HCAA	**5.185**	**4.182**	**1.058**	**3.980**	**4.088**	**0.902**	**ND**	**4.882**	**0.668**	**4.171**	**3.620**	**2.898**	**1.184**
Flavonoids													
Acacetin 7-*o*-glucuronide	0.530 ± 0.091	0.345 ± 0.00	ND	ND	0.361 ± 0.004	0.335 ± 0.001	0.322 ± 0.002	0.444 ± 0.050	0.291 ± 0.001	0.304 ± 0.00	0.305 ± 0.012	0.293 ± 0.010	0.384 ± 0.00
Apigenin-7-*o*-glucuronide	1.728 ± 0.092	1.362 ± 0.044	0.514 ± 0.010	1.214 ± 0.0116	1.030 ± 0.112	ND	ND	1.668 ± 0.034	0.290 ± 0.002	1.383 ± 0.102	1.427 ± 0.032	0.632 ± 0.050	0.370 ± 0.005
Chrysoeriol-8-*c*-glucoside	3.204 ± 0.644	2.576 ± 0.060	0.385 ± 0.00	2.555 ± 0.392	1.534 ± 0.047	ND	ND	3.849 ± 0.156	0.321 ± 0.004	2.660 ± 0.340	ND	0.791 ± 0.014	0.545 ± 0.008
Vitexin-2″-*o*-rhamnoside	4.847 ± 0.458	3.068 ± 0.359	0.815 ± 0.013	3.050 ± 0.350	4.056 ± 0.211	ND	ND	4.169 ± 0.138	0.558 ± 0.005	0.440 ± 0.007	0.547 ± 0.030	2.769 ± 0.440	1.064 ± 0.140
Cannflavin B	0.672 ± 0.044	0.454 ± 0.002	0.297 ± 0.00	0.413 ± 0.007	0.622 ± 0.010	0.396 ± 0.004	0.311 ± 0.003	0.712 ± 0.023	0.415 ± 0.005	0.655 ± 0.014	0.451 ± 0.018	0.528 ± 0.015	0.413 ± 0.016
Total flavonoïdes	**10.981**	**7.805**	**2.011**	**7.232**	**7.603**	**0.731**	**0.633**	**10.842**	**1.875**	**5.442**	**2.730**	**5.040**	**2.776**
Lignanamides													
Cannabisin A	1.713 ± 0.172	0.997 ± 0.012	ND	0.900 ± 0.022	1.445 ± 0.022	0.251 ± 0.021	ND	1.759 ± 0.040	0.196 ± 0.008	1.510 ± 0.201	0.934 ± 0.047	1.068 ± 0.091	ND
Total lignanamides	**1.713**	**0.997**	**ND**	**0.900**	**1.445**	**0.251**	**ND**	**0.759**	**0.196**	**1.510**	**0.934**	**1.068**	**ND**
Cannabinoids													
Sesqui CBGA	0.125 ± 0.023	0.086 ± 0.006	0.011 ± 0.001	0.082 ± 0.007	0.050 ± 0.001	ND	ND	ND	ND	ND	0.013 ± 0.00	ND	ND
CBTA isomer 1	0.052 ± 0.018	0.016 ± 0.00	ND	0.022 ± 0.00	0.043 ± 0.008	0.034 ± 0.00	0.040 ± 0.002	0.043 ± 0.006	0.042 ± 0.006	0.045 ± 0.002	0.022 ± 0.00	0.033 ± 0.004	0.051 ± 0.001
CBEA	0.010 ± 0.001	ND	ND	ND	ND	ND	0.0019 ± 0.00	0.007 ± 0.00	0.005 ± 0.00	0.009 ± 0.00	ND	ND	0.008 ± 0.00
CBTA isomer 2	0.026 ± 0.00	0.018 ± 0.00	ND	0.026 ± 0.00	0.019 ± 0.00	0.021 ± 0.001	0.018 ± 0.001	0.031 ± 0.00	0.026 ± 0.001	0.024 ± 0.00	0.029 ± 0.00	0.016 ± 0.001	0.037 ± 0.00
CBTA isomer 3	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND

Table 6 (*Contd.*)

Compounds	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11	Run 12	Run 13
						0.021 ± 0.00							
Δ9-THCMA isomer 1	ND	ND	ND	0.005 ± 0.00	ND	ND	ND	ND	0.032 ± 0.003	ND	ND	ND	ND
CBNDA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Δ9-THCMA isomer 2	0.241 ± 0.035	0.117 ± 0.012	ND	0.174 ± 0.003	0.113 ± 0.013	0.031 ± 0.002	0.017 ± 0.00	0.238 ± 0.094	0.015 ± 0.00	0.095 ± 0.017	0.296 ± 0.028	0.091 ± 0.022	0.060 ± 0.001
CBGMA isomer 1	ND	ND	ND	ND	ND	ND	ND	ND	0.023 ± 0.001	ND	ND	ND	ND
CBGMA isomer 2	0.008 ± 0.00	ND	ND	ND	0.008 ± 0.001	0.079 ± 0.00	ND	0.004 ± 0.00	0.012 ± 0.00	0.015 ± 0.00	ND	ND	ND
CBDA	2.183 ± 0.331	0.791 ± 0.042	ND	0.932 ± 0.055	2.267 ± 0.008	2.216 ± 0.019	2.224 ± 0.096	2.061 ± 0.080	2.359 ± 0.064	2.284 ± 0.068	1.163 ± 0.002	1.634 ± 0.091	2.386 ± 0.035
CBD	0.044 ± 0.005	0.018 ± 0.002	0.008 ± 0.00	0.021 ± 0.001	0.047 ± 0.00	0.047 ± 0.009	0.056 ± 0.001	0.051 ± 0.003	0.057 ± 0.006	0.052 ± 0.00	0.023 ± 0.00	0.023 ± 0.00	0.051 ± 0.007
Δ9-THCVA	0.126 ± 0.006	0.052 ± 0.013	ND	0.045 ± 0.008	0.106 ± 0.004	0.123 ± 0.023	0.116 ± 0.00	0.105 ± 0.006	0.117 ± 0.010	0.116 ± 0.001	0.039 ± 0.005	0.077 ± 0.015	0.117 ± 0011
CBDVA	0.103 ± 0.014	0.017 ± 0.00	ND	ND	0.074 ± 0.001	0.011 ± 0.00	0.056 ± 0.001	0.197 ± 0.008	0.010 ± 0.00	0.367 ± 0.075	ND	0.071 ± 0.004	0.026 ± 0.00
CBCVA	0.051 ± 0.00	ND	ND	ND	0.050 ± 0.005	0.055 ± 0.017	0.061 ± 0.002	0.043 ± 0.002	0.037 ± 0.002	0.124 ± 0.020	ND	0.047 ± 0.006	0.046 ± 0.0025
CBNA	0.088 ± 0.004	0.041 ± 0.009	ND	0.026 ± 0.00	0.044 ± 0.00	0.018 ± 0.00	0.053 ± 0.005	ND	0.035 ± 0.003	0.065 ± 0.001	0.055 ± 0.00	0.009 ± 0.002	0.043 ± 0.002
Δ9-THCA isomer 1	0.110 ± 0.021	ND	ND	0.063 ± 0.001	0.076 ± 0.003	0.075 ± 0.001	0.075 ± 0.009	0.080 ± 0.006	0.084 ± 0.015	0.068 ± 0.002	0.022 ± 0.00	0.066 ± 0.003	0.091 ± 0.003
Δ9-THCA isomer 2	3.647 ± 0.319	1.131 ± 0.051	0.005 ± 0.00	1.449 ± 0.158	3.560 ± 0.032	3.498 ± 0.052	3.555 ± 0.126	3.375 ± 0.056	3.727 ± 0.095	3.493 ± 0.129	0.678 ± 0.022	2.752 ± 0.133	3.736 ± 0.083
CBCA	0.977 ± 0.207	0.223 ± 0.012	ND	0.240 ± 0.021	0.052 ± 0.002	0.776 ± 0.012	0.766 ± 0.046	0.797 ± 0.017	0.829 ± 0.017	0.738 ± 0.070	0.241 ± 0.022	0.560 ± 0.045	0.831 ± 0.012
CBLA	0.593 ± 0.045	0.020 ± 0.004	ND	0.134 ± 0.00	0.360 ± 0.019	0.040 ± 0.00	0.047 ± 0.006	0.716 ± 0.013	0.059 ± 0.006	0.954 ± 0.024	0.109 ± 0.006	0.254 ± 0.008	0.085 ± 0.018
Total cannabinoids	**8.393**	**2.535**	**0.025**	**3.225**	**6.930**	**6.981**	**7.095**	**7.751**	**7.419**	**8.452**	**2.638**	**5.638**	**7.564**

solvent mixture produced a similar qualitative profile, although with lower levels Δ9-THCA and CBDA (3.736 and 2.386 mg CBNE per g DWL, respectively). In contrast, pure water (Run 3) extracted minimal concentration of cannabinoids 0.025–0.272 mg CBNE per g DW from leaves and inflorescence respectively in both matrices. Concerning CBD, it was detected at comparatively low levels in both matrices. CBD concentrations varied between 0.055 to 0.205 mg CBNE per g DWI in inflorescences whereas and from 0.008 to 0.057 mg CBNE per g DWL in leaves, confirming the predominance of acidic cannabinoids in dried plant material. The low CBD content suggests limited decarboxylation because no thermal treatment was applied during extraction. These findings also highlight that ethanol–acetone mixtures are more effective than pure water for cannabinoid recovery. In agreement with a previous study, ethanol is recognized as an efficient solvent for cannabinoid extraction from cannabis inflorescences.[31] Moreover, Brighenti *et al.* reported that CBDA was the most abundant compound in cannabis inflorescence samples extracted with ethanol, ranging from 0.1 to 46.8 mg g^{-1}, whereas CBD was the second most abundant cannabinoid (0.1–23.9 mg g^{-1}), with a concentration 4 to 10 times lower than that of CBDA. This confirms that cannabinoids are biosynthesized mainly as acids in cannabis and that decarboxylated compounds generally occur at lower levels.[32]

Pure water (Run 3) was ineffective for cannabinoid extraction because the chemical proprieties of these compounds. Despite having a carboxyl group (–COOH), the long terpenoids chain and aromatic ring of acidic cannabinoids make them relatively lipophilic.[26] Therefore, water alone cannot efficiently solubilize these hydrophobic molecules, resulting in low recovery. Moreover, matrix differences between inflorescences and leaves lead to the different cannabinoid accumulation patterns. Inflorescences contain a high density of glandular trichomes, which are the primary sites of cannabinoid biosynthesis and storage. Consequently, cannabinoid levels are lower in leaves, which primarily perform photosynthesis and transpiration.[37,38]

HCAA also showed matrix- and solvent-dependent distribution. In inflorescences, total HCAA content ranged from 0.295 to 3.033 mg CTE per g DWI, with a maximum reached in Run 11 (2/3 water–1/3 acetone). Leaves contained higher amounts, reaching 5.185 mg CTE per g DWL in Run 1 (1/3 water 2/3 ethanol). *N-trans*-Coumaroyltyramine was the predominant HCAA in both matrices. This observation is in agreement with Muller *et al.*, who identified several phenolic compounds, particularly *N-trans*-coumaroyltyramine, as major HCAA in leaves and inflorescences of *Cannabis sativa* L.[28] Due to their chemical nature, HCAA were preferentially extracted by more polar solvent systems (Run 1 with 33% water and Run 11 with 66% water for leaves and inflorescences respectively). Indeed, the presence of phenolic hydroxyl and amide groups may promote hydrogen bonding, thereby enhancing their solubility in polar solvents. The improved recovery of HCAAs and lignanamides by mixed solvent systems can also be explained by the complementary physicochemical properties of water, ethanol and acetone. Water promotes hydration and swelling of the plant matrix, thereby facilitating solvent penetration, while ethanol and acetone modulate the overall polarity of the extraction medium and enhance the solubility of less polar aromatic moieties. Thus, the combination of these solvents provides a broader and more flexible polarity range, which may be better suited for the extraction of compounds with both polar functional groups and aromatic moieties, such as HCAAs and lignanamides.[7,15] Additionally, the difference of concentration between the two matrices probably reflects their role in plant defense against abiotic and biotic stresses responses. As organs directly exposed to external environmental conditions, leaves tend to accumulate higher levels of HCAA which contribute to stress adaptation through their antioxidant properties.[34,39,40]

Phenolic acids were present at modest levels, with higher concentrations in leaves (0.232 mg FAE per g DWL) in Run 1 than in inflorescences (0.119 mg FAE per g DWI in 1/3 water–1/3 ethanol–1/3 acetone mixture). Additionally, they were not detected in several solvent mixtures, particularly those characterized by lower polarity, which may reflect their polar chemical structure.

Flavonoids were also detected in both plant parts, with vitexin-2″-*O*-rhamnoside as the predominant compound and moderate quantitative variation between matrices. Unlike cannabinoids, flavonoids were more abundant in vegetative leaves than in inflorescences. Solvent mixture also strongly influenced flavonoid extraction. Run 1 (1/3 water–2/3 ethanol) produced the highest yields. Total flavonoids in leaves reached at 10.981 mg QE per g DWL, whereas a lower value was recorded in inflorescences (5.297 mg QE per g DWI). Vitexin-2″-rhamnoside was the most abundant flavonoid in both matrices, reaching 1.930 and 4.847 mg g^{-1} in inflorescences and leaves, respectively. These findings suggest that ethanol and water-rich solvent mixtures improve the extraction of flavonoids. In addition, there was a noticeable difference between the plant matrices: leaves accumulated more flavonoids than inflorescences, which is consistent with the physiological function of leaves and their higher concentration of polar phenolic compounds. Moreover, Vitexin derivatives are preferentially extracted by water–ethanol mixtures, which contains several hydroxyl groups and sugar moieties. These characteristics promote strong hydrogen bonding with polar solvents, particularly water, whereas ethanol enhances solubilization of the flavonoid skeleton.[41] For the various phytochemical classes, such as flavonoids, HCAAs, phenolic acids, and lignanamides, the 1/3 water–2/3 ethanol solvent mixture demonstrated a high extraction capacity, particularly from leaves. These substances have a substantial contribution to radical scavenging (DPPH, ABTS) and reducing power tests, especially FRAP, because they act as efficient hydrogen or electron donors. Therefore, the increased antioxidant activity observed for this mixture is probably related to the higher flavonoid content.

These findings demonstrate the strong influence of solvent composition and plant tissue on phytochemical recovery, highlighting the importance of solvent interactions because no single solvent maximized the extraction of all chemical classes. Cannabinoids were the most abundant class, reaching a maximum value of 10.56 mg CBNE per g DWI with the 1/6 water–1/6 ethanol–2/3 acetone mixture, while phenolic acids

Table 7 Predictive and observed values for inflorescence extract at optimal conditions (33% water, 33% ethanol, 33% acetone)

Responses	Observed values (mean)	Predicted values	Confidence intervals at 95%
DPPH (mg TE per g inflorescence)	4.99 ± 0.01	4.64	3.42–5.85
ABTS (mg TE per g inflorescence)	8.92 ± 0.0045	8.13	7.02–9.23
FRAP (mg TE per g inflorescence)	30.36 ± 1.42	38.67	9.58–67.75
CUPRAC (mg TE per g inflorescence)	10.79 ± 1.25	14.15	4.59–23.71
TAC (mg TE per g inflorescence)	14.04 ± 0.73	11.83	5.91–17.76
TPC (mg GAE per g inflorescence)	14.10 ± 0.58	14.55	6.17–16.93

were present at low levels (0.119 mg FAE per g DWI) and HCAAs showed moderate extraction (3.03 mg CTE per g DWI). Each class's polarity and molecular structure can account for these variations. Cannabinoids such as Δ9-THCA and CBDA possess a large hydrophobic terpenoids moiety and few polar functional groups, making them relatively non-polar.[33] As a result, they are more soluble in organic solvents of intermediate polarity, like acetone, which can interact with both hydrophobic and moderately polar regions. In contrast, HCAAs have intermediate polarity due to the presence of amide and hydroxyl groups.[34] Therefore, mixed solvents are required for efficient solubilization. Phenolic acids are smaller and more polar compounds, containing hydroxyl and carboxylic groups,[42] which increases their affinity for aqueous phases but limits their extraction in acetone-rich systems. The maximum TPC observed for inflorescences with the acetone-rich mixture (16.05 mg GAE per g DWI) reflects the recovery of phenolic compounds of intermediate polarity. Additionally, the antioxidant activities followed similar trends and were strongly associated with both the amount and chemical nature of the extracted compounds. Phenolic compounds, especially HCAAs and other polyphenols, can act as effective hydrogen or electron donors because of their hydroxyl groups,[42] explaining their contribution to DPPH and ABTS radical-scavenging assays. In addition, conjugated phenolic and some cannabinoids may transfer electrons and stabilize redox intermediates, making them responsive in FRAP and CUPRAC assays.[20] This may explain the high FRAP value (52.79 mg TE per g DWI) observed for the acetone-rich mixture. In leaves, polar compounds contributed more strongly, enhancing antioxidant activity in solvent regions containing water, particularly in radical-scavenging assays. Overall, the interaction between plant matrix and solvent composition strongly influenced phytochemical extraction and antioxidant activity. Combining solvents increased overall antioxidant performance by broadening the diversity of extracted compounds. Therefore, a multi-response optimization approach was applied to identify the solvent mixture that maximized all studied responses.

3.4 Optimization of extraction and model validation

All responses (TPC, TAC, DPPH, ABTS, FRAP, and CUPRAC) were simultaneously optimized by maximizing the overall desirability function. The optimal solvent mixture for phenolic extraction from inflorescences was 33% water, 33% ethanol, and 33% acetone, whereas the optimal mixture for leaves was 50% water, 30% ethanol, and 20% acetone. All experimental response values fell within their corresponding confidence intervals (Tables 7 and 8), confirming the validity and acceptable predictive accuracy of both models.

Under the optimized conditions, total phenolic contents reached 14.10 and 12.48 mg GAE per g DW for inflorescences and leaves, respectively. Several studies have similarly investigated the influence of solvent composition on the extraction of bioactive compounds from cannabis and have often identified water/ethanol mixtures as suitable binary systems. For example, Drinić *et al.* reported that 50% ethanol/water was effective for phenolic extraction, yielding 9.25 and 17.05 mg g^{-1} from mature and young hemp, respectively.[22] In the same context, Cao *et al.* also optimized total flavonoid extraction from hemp leaf powder using different ethanol proportions.[43] Other studies optimized extraction conditions using response surface methodology for total phenolic recovery from cannabis leaves with water as a green solvent (19.08 mg g^{-1} DW) and from cannabis residues with 25% ethanol and 75% methanol, highlighting the effectiveness of polar solvent combinations in increasing phenolic content and antioxidant activity.[5,6]

Recent studies on other medicinal plants also support the use of mixture designs for extraction optimization. Soussi *et al.* reported that a ternary mixture of 44% water, 22% ethanol, and

Table 8 Predictive and observed values for leaf extract at optimal condition (50% water, 30% ethanol, 20% acetone)

Responses	Observed values (mean ± SD)	Predicted values	Confidence intervals at 95%
DPPH (mg TE per g leaves)	5.75 ± 0.05	5.62	5.10–6.13
ABTS (mg TE per g leaves)	8.83 ± 0.01	8.79	8.39–9.18
FRAP (mg TE per g leaves)	45.42 ± 1.91	52.07	43.02–61.12
CUPRAC (mg TE per g leaves)	7.61 ± 0.27	8.06	6.57–9.54
TAC (mg TE per g leaves)	10.21 ± 0.85	13.06	7.89–18.24
TPC (mg GAE per g leaves)	12.48 ± 0.24	12.41	11.46–13.37

34% methanol was optimal for extracting phenolic and flavonoid compounds from *Pimpinella anisum* and enhancing DPPH antioxidant activity.[44] Similarly, Šain *et al.* used response surface methodology coupled with a Box–Behnken design to evaluate the effect of several parameters, including the ethanol/water ratio, on phenolic extraction from industrial hemp. They found that 25% ethanol/water was the most effective mixture for enhancing FRAP and DPPH activities, reaching 0.059 Fe^{2+} equivalents per g DM and 0.361 mmol Trolox per g DM, respectively.[45] For cannabis leaves, Karnsinee *et al.* optimized microwave-assisted extraction of bioactive compounds using RSM and found that water significantly enhanced antioxidant activity, particularly in ABTS and FRAP assays (44.14 and 42.09 mg Trolox per g DM, respectively).[5] Overall, the present study confirms that mixture design is a powerful tool for optimizing the antioxidant potential of cannabis extracts.

Although solvent composition played a key role in bioactive compound recovery, the plant matrix was also an important factor. The different optimal solvent mixtures for leaves and inflorescences can be explained by differences in their chemical profiles, as shown by semi-quantification of phenolic compounds. Inflorescences contained higher cannabinoid levels and a broad range of compounds with low concentrations and different polarities, which may explain the need for a solvent mixture with equal proportions of the three solvents. In contrast, leaves were richer in polar phenolic compounds, especially hydroxycinnamic acid amides and phenolic acids, favoring a solvent mixture with a higher water proportion (50%).

4 Conclusion

The chemical diversity of *Cannabis sativa* metabolites has stimulated extensive research into their extraction and characterization. In this study, water, ethanol, and acetone were combined to enhance the extraction of phenolic compounds and antioxidants from *Cannabis sativa* L. leaves and inflorescences. Multi-response optimization showed that each plant matrix required a specific optimal solvent mixture, confirming matrix-dependent extraction behavior: 1/3 water–1/3 acetone–1/3 ethanol for inflorescences and 50% water–30% ethanol–20% acetone for leaves. HPLC-DAD/ESI-MS^2 enabled the tentative identification of several compound classes, including cannabinoids, hydroxycinnamic acid amides, flavonoids, and lignanamides. Inflorescences were characterized by higher total cannabinoid contents, whereas leaves contained higher levels of hydroxycinnamic acid amides. These differences highlight both the importance of solvent selection and the distinct chemical composition of *Cannabis sativa* L. organs. Overall, these results support the potential valorization of the optimized *Cannabis sativa* leaf and inflorescence extracts as sources of antioxidant and bioactive compounds for prospective applications in natural cosmetic formulations, such as antioxidant creams, serums or other topical skincare products, as well as in nutraceutical formulations, including functional supplements enriched with plant-derived antioxidants. However, additional studies on formulation stability, bioavailability, efficacy, and safety are required before practical application.

Author contributions

Omayma El Maabiche: experiment, formal analysis, software, writing – original draft. Chaymae Benkirane: formal analysis, writing – original draft. Rafika El Ati: formal analysis, validation. Yamina Haddad: data curation, software. Marie-Laure Fauconnier: experiment, writing – review & editing. Larbi Allai: validation, writing – review & editing, Farid Mansouri: conceptualization, methodology, funding acquisition, project administration, writing – review & editing, supervision.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data supporting the findings of this study are available from the corresponding author upon request.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d6ra05874d.

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