International Journal of Food Science + Technology

International Journal of Food Science and Technology 2023, 58, 1367–1381

Original article

Phenolic profiles of non-industrial hemp (*Cannabis sativa* L.) seed varieties collected from four different Moroccan regions

Chaymae Benkirane,[1] Farid Mansouri,[1,2]* Abdessamad Ben Moumen,[1] Yassine Taaifi,[1] Reda Melhaoui,[1] Hana Serghini Caid,[1] Marie-Laure Fauconnier,[3] Ahmed Elamrani[1] & Malika Abid[1]

1 Laboratory of Agricultural Production Improvement, Biotechnology and Environment, Faculty of Sciences, Mohammed I University, B.P. 717, Oujda 60000, Morocco

2 Higher School of Education and Training, Mohammed I University, B.P. 410, Oujda 60000, Morocco

3 Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, Gembloux 5030, Belgium

(Received 15 October 2022; Accepted in revised form 4 January 2023)

Summary This study aims to characterise the seeds of two varieties (Beldia and Critical) of hemp grown in four geographical areas of northern Morocco regarding their phenolic compounds and antioxidant power. Thirty-three phenolic compounds were identified using the HPLC-DAD/ESI-MS^2 technique. The phenolic profiles of hemp seeds of all varieties from all regions were characterised by their richness in *N-trans*-caffeoyltyramine (390.22–721.41 μg g^{-1}), cannabisin A (217.96–393.37 μg CTE g^{-1}) and cannabisin B (195.25–331.28 μg CTE g^{-1}). The antioxidant activities (expressed as IC_{50} and EC_{50} values) were 1.83–4.14, 1.64–4.37, 2.45–6.02, 2.65–9.29 and 1.75–4.37 mg mL^{-1} of extract for the TAC, DPPH, ABTS, CUPRAC and FRAP tests respectively. A two-way analysis of variance showed that phenolic compounds' content was mainly related to the geographical location and its interaction with the genotype factor. Multivariate analysis showed that hemp seeds from the Jebha and Galaz regions were characterised by a high level of phenolic compounds and a potent antioxidant activity compared to Tamorot and Ratba regions. This characterisation constitutes an interesting database for breeders to create new varieties that meet fluctuating expectations of the cannabis industry.

Keywords Antioxidant activity, *Cannabis sativa* L., chemometrics, phenolic compounds, seeds.

Introduction

Hemp (*Cannabis sativa* L., Cannabaceae) is a dioecious plant with a worldwide geographical distribution thanks to its great adaptability. It was domesticated, cultivated and used since antiquity for its fibre and medicinal properties. However, its cultivation has been prohibited since its use for psychoactive purposes related to the psychotropic Δ^9-tetrahydrocannabinol molecule, commonly known as THC (Schluttenhofer & Yuan, 2017). This molecule belongs to the cannabinoid family, representing the uniqueness of this plant. THC is accumulated in the glandular trichomes in the inflorescences of female plants, while it is scarcely present in male plants, stems and leaves. In contrast, roots and seeds are entirely devoid of THC (Glivar *et al.*, 2020). Over 100 other cannabinoids have been identified in cannabis, including cannabidiol (CBD) and cannabinol (CBN), which have no psychoactive effects (Pollastro *et al.*, 2018). Cannabis can be classified into two main chemotypes based on its THC/CBD ratio. By consensus, industrial cannabis has a low THC content (≤0.2%) with a THC/CBD ratio lower than 1, while non-industrial cannabis, also called drug type, has high THC values (>0.2%) with a ratio greater than 1 (Cattaneo *et al.*, 2021).

Cannabis is a versatile plant. It is used extensively in several fields, such as the manufacture of textiles, paper and rope, the insulation of buildings and the preparation of medicines and some cosmetic products (Crini *et al.*, 2020). The cannabis industry has thrived even more after using its seeds as animal feed and functional food for humans. Indeed, several studies have highlighted the nutritional importance of cannabis seeds due to their richness in high-quality proteins, fibres, carbohydrates and polyunsaturated fatty acids (Farinon *et al.*, 2020; Leonard *et al.*, 2020a). Hemp seeds are also known for their high content of bioactive compounds, mainly phenolic compounds.

*Correspondent: E-mail: f.mansouri@ump.ac.ma

doi:10.1111/ijfs.16298

Phenolic compounds are ubiquitous substances in the plant kingdom and are produced by the plant's secondary metabolism. They play a vital role due to their various biological activities, which have been widely discussed in several reviews (Huang *et al*., 2009; Mutha *et al*., 2021). Cannabis seeds are recognised for their abundance of phenylpropanoids, a particular phenolic class that comprises hydroxycinnamic acid amides (HCA) and lignanamides. HCA are associations between phenolic acids and amines through an amide bond (Roumani *et al*., 2020). They constitute the monomeric intermediates for the biosynthesis of lignanamides by oxidative coupling mechanisms (Leonard *et al*., 2020b).

Several factors affect the phenolic compound content of hemp seeds, such as the genetic heritage of the variety, growing environment, storage conditions and extraction techniques (Flores-Sanchez & Verpoorte, 2008; Isidore *et al*., 2021).

For historical, cultural and recent legislative reasons, the cultivation of cannabis in Morocco was and still is restricted to the Rif Mountains, where we find mainly non-industrial cannabis cultivated for recreational purposes to produce cannabis resin. There are local ecotypes (biotypes) named 'Beldia', which means 'local' or 'indigenous', as opposed to other hybrid exogenous varieties such as Critical, Pakistana and Mexicana, introduced from the Netherlands, Pakistan and Mexico respectively (Chouvy & Afsahi, 2014).

The objective of this study was to characterise and compare the phenolic profile and the antioxidant power of non-industrial hemp (*Cannabis sativa* L.) seeds collected from varieties grown in four regions in northern Morocco. To our knowledge, this work is the first to compare the phenolic profile, shedding light on the effect of genotype, geographical location and their interaction on the phenolic compounds identified in the seeds of cannabis grown in Morocco. In previous work from our research group, we characterised hemp seed varieties based mainly on their oil content, fatty acids, tocopherols and triacylglycerol composition (Taaifi *et al*., 2021). The present work concerns hemp seeds' phenolic content and antioxidant activities for more in-depth and complete characterisation.

The lack of data on the phenolic profile of hemp seeds from varieties grown in Morocco underlines the scientific importance of this study. Furthermore, this study is of great economic and social importance since it encourages enhancing this non-cannabinoid part of the plant (seeds) to help the local population bring added value to their crop. The characterisation of Moroccan hemp seeds also contributes to elaborating an interesting and valuable database for breeders to create new varieties that meet fluctuating expectations of the cannabis industry.

Materials and methods

Sampling site and seed material

Seven cannabis populations were studied in this work. These populations belong to two varieties, Beldia and Critical, which are the most cultivated in Morocco. The seeds were collected from four different areas of northern Morocco: Galaz, Jebha, Ratba and Tamorot, which are the main areas of cannabis cultivation and have distinct environmental characteristics. Beldia seeds were collected from all four sites, while Critical seeds were only collected from Jebha, Tamorot and Ratba. The four regions' geographical coordinates, altitude and climatic conditions (precipitations, maximum, minimum and average temperature) are listed in Table S1 (Supplementary data).

A total of 21 samples were collected, considering three biological replicates for each population (7 populations × 3 replicates). Cannabis plants of the studied populations were cultivated in the spring of 2019. At maturity, when the colour of more than 90% of the seeds turns brown (July for Beldia and September for Critical), the cannabis plants were harvested and sundried for 3 to 5 days. Then, the seeds were collected, cleaned and stored at 4 °C until use.

Extract preparation

The crushed seeds were first defatted by Soxhlet using petroleum ether for 5 h to eliminate fat, which could interfere with the phenolic compounds. The obtained defatted seeds were used to prepare extracts using 50% aqueous acetone with a solid:liquid ratio of 1:10 (Benkirane *et al*., 2022). The extraction was carried out by vortexing the mixture for 5 min and sonicating it for 45 min in an ultrasound bath (Transonic T460, Germany) in a darkened cold room. After centrifugation, the pellet was re-extracted in the same way. The two supernatants were combined and evaporated using a rotary evaporator. For phenolic compound analysis, the dry extracts were resuspended in 2 mL of methanol and filtered (0.45 µm) before being injected into the HPLC system. For antioxidant activity evaluation, the dry extracts were resuspended to a concentration of 6 mg mL^{-1}, followed by a series of dilutions (5, 4, 3, 2, 1 and 0.5 mg mL^{-1}). The prepared extracts were stored at 4 °C until use.

Identification and quantification of phenolic compounds by HPLC-DAD/ESI-MS^2

The phenolic compounds were separated using an Agilent 1260 Infinity II high-performance liquid chromatography system (HPLC, Agilent Technologies, USA) coupled with a diode array detector (DAD). The injected volume was 10 μL of each extract with a flow rate of 0.6 mL min^{-1}. The elution was carried out on an Eclipse column XDB-C18 (150 × 4.6 mm internal diameter, 3.5 μm particle size; Agilent Technologies, USA) as described by Benkirane *et al.* (2022). Phenolic compound separation was monitored at 254, 280, 300 and 340 nm wavelengths, while the UV–visible spectra of each compound were plotted between 190 and 600 nm. The chromatograms were visualised and analysed using the Agilent Chemstation 32 software.

At the output of the HPLC system, the separated peaks were recovered individually in 2 mL vials and analysed by mass spectrometry (Esquire HCT mass spectrometer, Bruker Daltonics, Germany) in negative and positive modes. The samples were ionised by electrospray ionisation (ESI) source using smart mode with a target mass of 400, 500 and 600 m/z, setting the following parameters: spray voltage 4500 V, dry gas temperature 200 °C, nebuliser 10 psi and dry gas 4 L min^{-1}. The precursor ions were trapped in the ion trap, experiencing collision energy of 1–10% arbitrary units and expelled according to their m/z ratio. Mass spectra were acquired in ultra-scan mode using a mass range 50–1000 m/z at a speed of 26 000 m/z/s. ESI Tuning mix (Agilent Technologies, Santa Clara, *CA*., USA) was used to calibrate the instrument and the m/z scale. Instrument control and data acquisition were ensured *via* the Esquire Control software, while mass data processing was performed using the ACD/labs 2021.2.1 software.

Phenolic compounds were identified by comparing their molecular ion mass, fragment ions and UV spectrum with data from the literature and some mass databases, such as the MassBank of North America (MoNA). Phenolic acids and *N-trans*-caffeoyltyramine were also identified using commercial standards from Sigma-Aldrich (St-Louis, MO, USA).

The quantification of phenolic acids was carried out using their authentic commercial standards: benzoic acid (60–980 μg mL^{-1}, LOQ = 146 μg mL^{-1} and LOD = 48 μg mL^{-1}), sinapic acid (60–980 μg mL^{-1}, LOQ = 95 μg mL^{-1} and LOD = 32 μg mL^{-1}), ferulic acid (60–980 μg mL^{-1}, LOQ = 104 μg mL^{-1} and LOD = 34 μg mL^{-1}) and *p*-coumaric acid (75–1200 μg mL^{-1}, LOQ = 73 μg mL^{-1} and LOD = 24 μg mL^{-1}). The phenolic compounds from the other classes were semi-quantified using the *N-trans*-caffeoyltyramine standard curve (60–980 μg mL^{-1}, LOD = 34 μg mL^{-1} and LOQ = 102 μg mL^{-1}) due to the limited availability of standards. Results were then expressed as μg caffeoyltyramine equivalent per g of seeds (μg CTE g^{-1} seeds).

Determination of antioxidant activity

The antioxidant activity was evaluated by different spectrophotometric tests using a UV–Visible spectrophotometer (Jenway 7305, France). Total antioxidant capacity assay (TAC) was performed using the phosphomolybdenum method. Radical scavenging ability was evaluated using 2,2- diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals. The reducing power was estimated using cupric-reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays. All antioxidant assays were carried out as described in previously published works (Kadriye *et al.*, 2012; Grochowski *et al.*, 2017).

The results were expressed as IC_{50} (median inhibitory concentration) or EC_{50} (median effective concentration) values after normalising the measured absorbances and log-transforming the extracts' concentrations. The IC_{50} (used for the DPPH and ABTS tests) and EC_{50} (used for the TAC, FRAP and CUPRAC tests) values were calculated based on a dose–response model (non-linear regression) using the GraphPad Prism 9.4.0 software.

Multivariate analysis and statistical tests

All results were presented as mean ± standard deviation (SD). Data were statistically analysed using the general linear model procedure with a two-factor design, including variety, growing area and their interaction as sources of variation. The Galaz region was excluded from the interaction effect analysis due to the absence of the Critical variety in this region. Additionally, one-way ANOVA followed by Tukey's test was performed to compare between means of the seven cannabis populations. The significance level was set at 5%. Multivariate analysis techniques (principal component analysis and hierarchical clustering) were used to group individuals into homogeneous groups and characterise them according to the studied variables. All statistical tests were generated and processed using IBM SPSS Statistics 25 and JMP Pro 15 (SAS Institute Inc.) software.

Results and discussion

The phenolic profile of cannabis seeds

The phenolic compounds in hemp seed extracts of seven populations were identified by HPLC-DAD/ESI-MS^2 analysis. Four phenolic classes were identified in the studied cannabis varieties: phenolic acids, hydroxycinnamic acid amides (HCA), lignanamides and cannabinoids, totalling 33 compounds. Table 1 presents the retention time (min), molecular formula, experimental and theoretical molecular ion mass (m/z), error (ppm), fragment ions (m/z) with their relative abundance (%) and UV λ_{max} (nm) of the identified phenolic compounds in negative mode. The identification was carried out by comparing these spectral data

Table 1 HPLC-DAD/ESI-MS^2 identification of phenolic compounds (negative mode $[M - H]^-$) in hemp seed extracts of Beldia and Critical varieties cultivated in four regions of Morocco

N° peak	RT (min)	Compound name	Molecular formula	UV λ_{max} (nm)	Theoretical mass (m/z)	Experimental mass (m/z)	Error (ppm)	Mass fragments (% intensity)	References
1	13.9	Benzoic acid	$C_7H_6O_2$	222–285	122.0373	121.0375	1.65	not fragmented	MoNA
2	16.1	*p*-Coumaric acid	$C_9H_8O_3$	230–295–310	164.0478	163.0481	1.84	119 (100), 93 (8)	MoNA
3	17.7	Ferulic acid	$C_{10}H_{10}O_4$	225–292–315	194.0584	193.0588	2.07	134 (100), 178 (37), 149 (13)	MoNA
4	19.2	*N-trans*-caffeoyltyramine isomer	$C_{17}H_{16}NO_4$	220–280–315	299.1158	298.1161	1.01	135 (100), 178 (46), 298 (45), 161 (25), 136 (12), 284 (6)	(Nigro *et al.*, 2020)
5	20.8	*N-trans*-caffeoyltyramine	$C_{17}H_{16}NO_4$	220–290–320	299.1158	298.1159	0.34	135 (100), 178 (43), 161 (42), 136 (30), 298 (16), 256 (5), 148 (5)	(Nigro *et al.*, 2020)
6	23.4	Cannabisin A	$C_{34}H_{30}O_8N_2$	255	594.2002	593.2014	2.02	593 (100), 454 (27), 639 (21), 523 (16), 536 (13), 428 (11), 482 (11)	(Nigro *et al.*, 2020)
7	23.7	Cannabisin B	$C_{34}H_{32}O_8N_2$	225–250–285–310–335	596.2159	595.2148	−1.85	432 (100), 595 (93), 485 (68), 269 (33), 322 (30)	(Nigro *et al.*, 2020)
8	24.2	*N-trans*-coumaroyltyramine	$C_{17}H_{17}O_3N$	224–290–310	283.1208	282.1218	3.54	145 (100), 119 (85), 282 (54), 162 (46), 134 (14), 240 (10)	(Nigro *et al.*, 2020)
9	24.6	Cannabisin B isomer 1	$C_{34}H_{32}O_8N_2$	255–310	596.2159	595.2151	−1.34	416 (100), 595 (99), 269 (46), 432 (45), 458 (30), 485 (29), 295 (23)	(Nigro *et al.*, 2020)
10	24.9	*N*-feruloyltyramine	$C_{18}H_{19}O_4N$	220–292–318	313.1314	312.1322	2.56	178 (100), 135 (45), 297 (46), 312 (45), 148 (12)	(Nigro *et al.*, 2020)
11	25.3	Cannabisin B isomer2	$C_{34}H_{32}O_8N_2$	224–250–290–335	596.2159	595.2164	0.84	485 (100), 432 (40), 322 (33), 269 (20), 348 (9), 456 (8), 595 (6)	(Nigro *et al.*, 2020)
12	25.9	Demethylgrossamide	$C_{35}H_{34}N_2O_8$	225–250–285–330	610.2315	609. 2324	1.47	283 (100), 446 (66), 268 (26), 609 (9), 377 (4)	(Nigro *et al.*, 2020)
13	26.4	Cannabisin C	$C_{35}H_{34}O_8N_2$	220–245–292–320	610.2315	609. 2321	0.98	499 (100), 609 (95), 446 (70), 447 (27), 336 (20), 269 (5)	(Nigro *et al.*, 2020)
14	26.9	Cannabisin C isomer	$C_{35}H_{34}O_8N_2$	220–280–335–410	610.2315	609.2332	2.79	446 (100), 485 (66), 609 (21), 322 (19), 472 (7), 279 (6), 499 (6)	(Nigro *et al.*, 2020)
15	28.5	Cannabisin D	$C_{36}H_{36}N_2O_8$	225–250–285–340	624.2472	623.2479	1.12	460 (100), 623 (94), 283 (35), 268 (7), 444 (9), 499 (4)	(Nigro *et al.*, 2020)
16	28.9	3,3-Didemethylgrossamide	$C_{34}H_{32}N_2O_8$	225–290–324	596.2159	595.2168	1.51	432 (100), 269 (99), 458 (36), 595 (22), 295 (10), 338 (7), 250 (2)	(Nigro *et al.*, 2020)
17	29.1	Tri-p-coumaroylspermidine	$C_{34}H_{37}N_3O_6$	255	583.2688	582.2691	0.52	462 (100), 582 (87), 342 (76), 316 (10), 436 (11), 299 (4), 217 (2), 533 (2)	(Nigro *et al.*, 2020)
18	29.8	Cannabisin E	$C_{36}H_{38}N_2O_9$	220–283–316	642.2577	641.2579	0.31	623 (100), 489 (90), 281 (65), 431 (40), 641 (30), 591 (15), 460 (11), 312 (12)	(Nigro *et al.*, 2020)
19	30.2	Grossamide K	$C_{28}H_{29}NO_7$	225–288–325	491.1949	490.1957	1.63	472 (100), 490 (43), 460 (34), 488 (2)	(Nigro *et al.*, 2020)
20	31.3	Cannabisin M	$C_{34}H_{32}N_2O_8$	223–285–315	596.2159	595.2147	−2.04	298 (100), 595 (57), 430 (17), 427 (5), 547 (4)	(Nigro *et al.*, 2020)
21	31.5	3,3′-Demethyl-heliotropamide	$C_{34}H_{32}N_2O_8$	223–285–315	596.2159	595.2168	1.51	107 (100), 298 (49), 595 (23)	(Nigro *et al.*, 2020)
22	31.7	Unnamed condensed trilignanamide	$C_{51}H_{47}N_3O_{12}$	222–278–315	893.3160	892.3164	0.44	430 (100), 595 (85), 593 (55), 727 (24), 485 (11), 322 (11), 892 (9)	(Moccia *et al.*, 2020)
23	32.0	Cannabisin Q	$C_{34}H_{32}N_2O_8$	290–320	596.2159	595.2154	−0.84	298 (100), 595 (42), 296 (7), 178 (1)	(Nigro *et al.*, 2020)

Table 1 (*Continued*)

N° peak	RT (min)	Compound name	Molecular formula	UV λ_{max} (nm)	Theoretical mass (m/z)	Experimental mass (m/z)	Error (ppm)	Mass fragments (% intensity)	References
24	32.3	Cannabisin F	$C_{36}H_{36}N_2O_8$	225–290	624.2472	623.2478	0.96	460 (100), 623 (61), 297 (35), 486 (29), 352 (5)	(Nigro *et al.*, 2020)
25	33.4	Isocannabisin N	$C_{35}H_{34}N_2O_9$	225–294–312	610.2315	609.2298	−2.79	609 (100), 296 (41), 312 (25), 417 (17), 446 (5), 176 (4), 581 (3)	Leonard *et al.* (2021)
26	34.2	Grossamide	$C_{36}H_{36}N_2O_8$	226–285–322	624.2472	623.2485	2.08	623 (100), 460 (77), 591 (47), 297 (32), 471 (30), 551 (23), 432 (17), 486 (15), 428 (11), 282 (11)	(Nigro *et al.*, 2020)
27	34.5	Cannabisin O	$C_{54}H_{53}N_3O_{12}$	229–315	935.3629	934.3641	1.28	Not Fragmented	---
28	35.4	Unnamed lignanamide	---	228–312	---	589.2366	---	426 (100), 261 (10), 443 (8), 589 (7), 255 (7), 279 (5), 163 (5)	---
29	39.4	Dihydrocannabinol	$C_{21}H_{28}O_2$	272	312.2089	311.2092	0.96	293 (100), 311 (82), 223 (59), 275 (41), 201 (26), 235 (20), 171 (15)	(Moccia *et al.*, 2020)
30	40.7	Cannabidiol (CBD)	$C_{21}H_{30}O_2$	280	314.2246	313.2257	3.51	313 (100), 201 (96), 171 (36), 295 (34), 277 (26), 202 (14), 165 (6), 172 (5), 183 (2), 129 (0,5)	(Moccia *et al.*, 2020)
31	44.2	Cannabielsoic acid	$C_{22}H_{29}O_5$	280	374.2099	373.2105	1.61	205 (100), 329 (94), 311 (84), 373 (56), 271 (35), 173 (16), 259 (8)	(Frassinetti *et al.*, 2018)
32	45.2	Sinapic acid	$C_{11}H_{12}O_5$	275	224.0690	223.0697	3.13	225 (100), 223 (34), 195 (36), 125 (35), 179 (24), 221 (20), 163 (18), 206 (16), 164 (12), 155 (17)	(Rea Martinez *et al.*, 2020)
33	45.9	Cannabidiolic acid (CBDA)	$C_{22}H_{30}O_4$	224–270–310	358.2144	357.2141	−0.84	339 (100), 357 (21), 340 (19), 341 (11), 311 (7), 313 (5), 289 (3), 179 (2), 271 (1), 245 (1)	(Rea Martinez *et al.*, 2020)

Abbreviation: MoNA, MassBank of North America.

with those previously published in the literature (Moccia *et al.*, 2020; Nigro *et al.*, 2020; Rea Martinez *et al.*, 2020; Leonard *et al.*, 2021).

The phenolic acids identified in cannabis seed extracts were benzoic acid (**1**), ferulic acid (**2**), *p*-coumaric acid (**3**) and sinapic acid (**32**). Their detection was confirmed using authentic commercial standards. Compounds **4**, **5**, **8**, **10** and **17** (Supplementary Fig. S1) were recognised as HCA, with either tyramine or spermidine as the amine moiety. Compounds **4** and **5** with deprotonated ions at m/z 298.1161 and 298.1159, respectively, exhibited similar intensive fragment ions at m/z 135 (corresponding to the loss of tyramine) and 178 (corresponding to the cleavage at the N-Cα bond). They had a typical UV spectrum showing absorbance peaks around 220, 290 and 320 nm (Supplementary Fig. S2), suggesting that they were likely *N-trans*-caffeoyltyramine geometrical isomers.

Compound **8** showed $[M–H]^-$ ion at m/z 282.1218 in accordance with the molecular formula $C_{17}H_{17}NO_3$. The fragment ions at m/z 145 and 162 confirmed the coumaroyl and coumaramide moieties respectively. The ion at m/z 119 suggested the loss of the tyramine moiety (−163 u), providing sufficient evidence to identify this compound as *N-trans*-coumaroyltyramine (Nigro *et al.*, 2020). Similarly, compounds 10 and 17 with $[M–H]^-$ ions at m/z 312.1322 and 582.2691 were attributable to *N*-feruloyltyramine (Leonard *et al.*, 2021) and tri-*p*-coumaroylspermidine (Nigro *et al.*, 2020) respectively.

Regarding lignanamides, 20 compounds were identified in cannabis seeds, including cannabisin A (**6**), B (**7**, **9** and **11**), C (**13** and **14**), D (**15**), E (**18**), M (**20**), Q (**23**), F (**24**), O (**27**), isocannabisin N (**25**), demethylgrossamide (**12**), 3,3-didemethylgrossamide (**16**), grossamide K (**19**), 3,3-demethyl-heliotropamide (**21**), grossamide (**26**) and two other unnamed lignanamides (**22** and **28**). Compound **6** was assigned as cannabisin A. Its $[M-H]^-$ molecular ion at m/z 593.2014 provided intensive ion fragments at m/z 593, 454, 639 and 523, with a UV maximum absorbance at 255 nm, which is consistent with previous studies (Nigro *et al.*, 2020). Compound **7** displayed a deprotonated molecular ion at m/z 595.2148 ($C_{34}H_{32}O_8N_2$) and fragment ions at m/z 432, 595, 485, 269 and 322, which is consistent with the cannabisin B molecule. Two other compounds (**9** and **11)** were tentatively identified as geometrical isomers of cannabisin B since they showed similar spectral data with compound **7** (Supplementary Fig. S2). Two unnamed lignanamides (compounds **22** and **28**) were also detected in cannabis seeds. Compound **22** showed the deprotonated molecular ion at m/z 892.3164 and intensive fragment ions at m/z 430, 595 and 593. The $[M– H]^-$ ion of the compound under peak **28** was at m/z 589.2366 with an ion at m/z 426 as the base peak, consistent with a loss of tyramine moiety. At the end of the phenolic profile (Supplementary Fig. S1), four cannabinoids were identified: dihydrocannabinol (**29**), cannabidiol (**30**), cannabielsoic acid (**31**) and cannabidiolic acid (**33**).

Some previous studies identified other phenolic acids (vanillic acid, protocatechuic acid and 4-hydroxybenzoic acid) and other HCAs like caffeoyloctopamine, which is frequent in various hemp seed varieties. It is also important to mention that several flavonoids, such as quercetin and its derivatives, were also identified in the seeds of industrial cannabis (Nigro *et al.*, 2020; Rea Martinez *et al.*, 2020). However, our results showed the absence of this phenolic class in the analysed samples, which could be a particularity of non-industrial cannabis. Indeed, the plant invests more in synthesising cannabinoids than phenolics in drug-type varieties.

The chromatographic results showed that the phenolic profile of the seven cannabis populations follows the same pattern. The difference between them lies in the relative content of each identified phenolic compound. We note that *N-trans*-coumaroyltyramine was detected in the Beldia variety of the Jebha region only, while it was absent in the others. Some cannabinoids were also not detected in cannabis seed extracts, such as cannabidiolic acid (CBDA), which was absent in the Critical variety from all regions (Table 2). However, it was suggested that cannabinoids are probably not synthesised in seeds but could be residues from aerial parts during seed cleaning (Ning *et al.*, 2022).

The results showed that cannabis seeds are rich in phenylpropanoids, including HCA and lignanamides. Nevertheless, phenolic acids and cannabinoids were detected in low quantities. Table 2 represents the content of each phenolic compound identified in the seven studied cannabis seed samples. Total identified phenols varied considerably among samples ranging from 2238.22 to 3242.46 μg g^{-1} seeds for Beldia Ratba and Beldia Jebha respectively.

Regarding phenolic acids, their total content oscillated from 157.15 to 270.43 μg g^{-1}. Benzoic acid was the most representative of this class in all varieties, with a content ranging from 83.84 in Critical Ratba to 180.83 μg g^{-1} in Beldia Jebha. The concentration of total HCA in cannabis seeds varied considerably from 695.79 for Beldia Ratba to 1171.88 μg CTE g^{-1} for Beldia Jebha. For lignanamides, their content ranged from 1203.45 for Critical Tamorot to 1772.21 μg CTE g^{-1} for Beldia Galaz. As for cannabinoids, they fluctuated from 23.3 to 97.53 μg CTE g^{-1} for Beldia Ratba and Beldia Tamorot respectively.

All varieties showed an abundance of *N-trans*-caffeoyltyramine, which constitutes the major phenolic compound of the analysed seeds (390.22 to 721.41 μg g^{-1}), followed by cannabisins A (217.96 to 393.37 μg CTE g^{-1}) and B (195.25 to 331.28 μg CTE

Table 2 Quantification (Mean ± SD) of the identified phenolic compounds in hemp seed extracts of Beldia and Critical varieties cultivated in four regions of Morocco

	Beldia				Critical				
Phenolic compounds	**Jebha**	**Tamorot**	**Ratba**	**Galaz**	**Jebha**	**Tamorot**	**Ratba**	**Min**	**Max**
Benzoic acid	143.71 ± 9.62b	176.83 ± 14.91a	150.51 ± 5.38b	180.30 ± 20.95a	106.85 ± 8.34c	135.00 ± 1.21b	83.85 ± 8.20d	75.64	222.33
p-Coumaric acid	5.54 ± 1.11cd	12.31 ± 0.32a	4.08 ± 0.76d	8.53 ± 3.77b	4.46 ± 1.51d	15.02 ± 0.98a	8.11 ± 2.62bc	1.48	16.04
Ferulic acid	52.08 ± 4.48ab	49.92 ± 0.54b	39.18 ± 0.52c	56.18 ± 3.60a	52.00 ± 6.27ab	41.29 ± 3.23c	37.08 ± 1.04c	35.45	65.53
Sinapic acid	24.68 ± 1.39d	30.27 ± 2.27b	ND	25.41 ± 0.27d	21.09 ± 0.45e	32.07 ± 0.40a	28.12 ± 0.75c	ND	32.80
Total phenolic acids	**226.00 ± 11.75b**	**269.33 ± 16.12a**	**193.77 ± 5.18c**	**270.43 ± 24.14a**	**184.39 ± 11.64c**	**223.38 ± 3.34b**	**157.15 ± 7.44d**	**148.79**	**322.28**
Caffeoyltyramine isomer	74.98 ± 16.99bc	88.59 ± 5.50ab	51.52 ± 1.26d	65.37 ± 16.16cd	103.46 ± 21.36a	53.49 ± 2.85d	66.96 ± 17.45cd	34.81	123.96
N-trans-caffeoyltyramine	721.41 ± 66.00a	472.16 ± 21.00bc	394.46 ± 83.97c	680.39 ± 128.67a	541.37 ± 23.57b	390.22 ± 16.55c	447.67 ± 36.90bc	201.52	903.48
N-trans-coumaroyltyramine	23.43 ± 1.39	ND	ND	ND	ND	ND	ND	ND	24.77
N-feruloyltyramine	165.62 ± 14.64a	128.05 ± 4.45bc	98.67 ± 9.16de	153.12 ± 48.89ab	90.14 ± 2.51e	114.50 ± 3.06cde	121.81 ± 4.41cd	86.44	247.32
Tri-*p*-coumaroylspermidine	186.43 ± 8.96cd	134.59 ± 0.57e	151.15 ± 4.40e	170.25 ± 18.33d	250.80 ± 9.76b	203.90 ± 22.48c	301.30 ± 12.26a	133.72	316.70
Total HCA	**1171.88 ± 92.01a**	**823.38 ± 21.05de**	**695.79 ± 88.45f**	**1069.13 ± 160.81ab**	**985.78 ± 42.03bc**	**762.11 ± 40.08ef**	**937.73 ± 34.97cd**	**501.89**	**1378.65**
Cannabisin A	344.47 ± 39.38b	257.10 ± 3.65de	276.91 ± 26.39cd	393.37 ± 68.05a	315.21 ± 12.24bc	217.96 ± 12.93e	305.50 ± 3.84bc	198.64	475.59
Cannabisin B	309.64 ± 31.18ab	218.88 ± 3.53cd	199.30 ± 17.18d	331.28 ± 49.60a	293.12 ± 12.37b	195.25 ± 11.85d	253.23 ± 5.26c	174.16	420.41
Cannabisin B isomer1	40.67 ± 1.68b	35.61 ± 0.70bcd	38.95 ± 2.36bc	47.87 ± 9.72a	31.11 ± 0.69d	30.48 ± 0.77d	34.02 ± 0.87cd	28.90	66.26
Cannabisin B isomer2	42.70 ± 2.51a	33.47 ± 1.02b	35.47 ± 1.34b	40.75 ± 3.12a	29.01 ± 2.19c	25.62 ± 0.85d	28.13 ± 0.31cd	24.22	46.30
Demethyl grossamide	36.45 ± 4.56a	27.42 ± 0.39b	27.34 ± 1.39b	38.41 ± 6.74a	28.34 ± 0.52b	25.95 ± 0.53b	24.01 ± 0.33b	23.54	47.88
Cannabisin C	136.44 ± 12.22a	98.33 ± 0.73de	104.91 ± 2.72cd	129.58 ± 19.99a	124.81 ± 4.72ab	85.11 ± 4.51e	115.85 ± 0.68bc	80.07	160.20
Cannabisin C isomer	53.44 ± 5.14a	37.76 ± 0.50e	39.28 ± 2.46de	47.72 ± 4.21b	43.65 ± 1.10c	35.74 ± 1.51e	43.03 ± 0.61cd	33.17	63.62
Cannabisin D	63.29 ± 4.46b	58.64 ± 1.20bc	54.71 ± 0.71cd	59.31 ± 7.13bc	76.40 ± 3.20a	50.36 ± 1.46d	78.43 ± 7.51a	46.19	90.99
3,3 Didemethylgrossamide	46.30 ± 5.78a	34.62 ± 0.48b	35.90 ± 3.17b	43.20 ± 8.33a	35.55 ± 2.57b	32.80 ± 1.93b	36.41 ± 5.40b	30.47	57.12
Cannabisin E	54.33 ± 3.44a	36.03 ± 4.22c	33.70 ± 0.53cd	43.04 ± 7.08b	23.56 ± 2.35e	31.10 ± 0.94cd	29.47 ± 1.88d	21.28	57.44
Grossamide K	44.18 ± 6.03a	30.29 ± 1.84b	29.51 ± 0.55b	40.08 ± 5.84a	44.23 ± 10.15a	36.77 ± 4.12ab	43.88 ± 0.54a	28.03	58.65
Cannabisin M	115.87 ± 21.15a	83.01 ± 0.49cd	90.33 ± 6.76bcd	114.29 ± 27.11a	102.02 ± 1.87abc	76.66 ± 3.85d	105.95 ± 5.28ab	72.75	161.53
3,3′ Demethylheliotropamide	42.54 ± 5.24a	33.11 ± 1.23b	35.77 ± 2.34b	42.67 ± 7.98a	34.99 ± 0.44b	31.03 ± 1.75b	44.96 ± 3.76a	28.78	59.32
Unnamed condensed trilignanamide	55.56 ± 8.20a	39.86 ± 0.33de	47.86 ± 3.45bc	54.96 ± 9.54ab	42.05 ± 0.79cde	36.84 ± 2.39e	46.06 ± 2.81cd	34.57	73.86
Cannabisin Q	47.52 ± 8.73a	35.83 ± 0.38c	36.87 ± 1.77c	44.58 ± 4.89a	44.55 ± 1.12a	37.50 ± 0.86bc	42.57 ± 0.67ab	34.35	65.24
Cannabisin F	36.51 ± 8.72ab	35.31 ± 0.84ab	28.32 ± 1.09c	31.69 ± 1.75bc	37.56 ± 0.38a	35.31 ± 1.79ab	40.53 ± 3.24a	19.97	53.72
Isocannabisin N	40.20 ± 8.50a	21.87 ± 0.56bc	22.12 ± 0.92bc	25.24 ± 0.81b	22.34 ± 0.26bc	23.73 ± 0.86bc	20.16 ± 0.18c	20.16	40.20
Grossamide	104.13 ± 4.18a	71.40 ± 1.95c	67.81 ± 1.73cd	86.09 ± 15.88b	58.87 ± 4.33d	69.36 ± 3.60c	72.65 ± 2.68c	51.65	108.82
Cannabisin O	78.13 ± 2.36b	63.94 ± 0.88cd	64.21 ± 0.67c	94.36 ± 6.76a	79.90 ± 2.76b	78.40 ± 3.80b	58.21 ± 6.20d	45.63	104.99
Unnamed lignanamide	62.53 ± 2.14a	58.66 ± 0.51b	56.10 ± 0.88c	63.74 ± 2.64a	53.01 ± 1.64d	47.47 ± 0.77e	41.84 ± 1.69f	39.00	68.78
Total Lignanamides	**1754.91 ± 114.75a**	**1311.16 ± 5.34cd**	**1325.36 ± 49.85c**	**1772.21 ± 165.63a**	**1520.29 ± 32.19b**	**1203.45 ± 30.38d**	**1464.89 ± 12.03b**	**1159.51**	**2071.15**
Total phenylpropanoids	**2926.79 ± 203.43a**	**2134.55 ± 23.46c**	**2021.15 ± 121.67c**	**2841.34 ± 304.90a**	**2506.07 ± 51.25b**	**1965.56 ± 61.31c**	**2402.62 ± 40.28b**	**1818.54**	**3449.79**
Dihydrocannabinol	17.38 ± 0.55d	19.62 ± 0.13b	ND	17.50 ± 0.30d	Tr	22.41 ± 0.07a	18.82 ± 0.36c	ND	22.55
Cannabidiol	16.13 ± 0.04c	16.00 ± 1.63c	ND	ND	Tr	21.86 ± 0.14a	18.34 ± 0.18b	ND	22.12
Cannabielsoic acid	31.39 ± 0.59d	39.12 ± 0.69a	23.30 ± 0.16e	33.94 ± 0.75b	32.78 ± 0.86c	38.76 ± 0.49a	38.59 ± 0.36a	22.99	40.13
Cannabidiolic acid (CBDA)	24.78 ± 0.25a	22.80 ± 0.42b	ND	17.64 ± 0.84c	ND	ND	ND	ND	24.99

Table 2 (*Continued*)

Phenolic compounds	Beldia				Critical				
	Jebha	Tamorot	Ratba	Galaz	Jebha	Tamorot	Ratba	Min	Max
Total Cannabinoids	89.67 ± 1.25b	97.53 ± 2.38a	23.30 ± 0.16g	69.08 ± 1.47e	32.78 ± 0.86f	83.02 ± 0.47c	75.75 ± 0.67d	22.99	100.40
Total Phenols	3242.46 ± 194.99a	2501.41 ± 18.82b	2238.22 ± 118.91c	3180.85 ± 326.26a	2723.24 ± 58.43b	2271.96 ± 62.23c	2635.52 ± 44.78b	2038.49	3843.66

Note: Different letters in the same line indicate significant differences ($P < 0.05$) between samples based on the Tukey test. HCAs, lignanamides and cannabinoids are expressed in µg caffeoyltyramine equivalent per g of seeds (µg CTE g^{-1} seeds). Phenolic acids are expressed in µg g^{-1} seeds.
Abbreviations: Nd, Not detected; Tr, Traces.

g^{-1}). This finding is in accordance with the results of several other studies, which report that these three substances are the most dominant in cannabis seeds (Frassinetti *et al.*, 2018; Leonard *et al.*, 2021) and even in other organs such as roots (Flores-Sanchez & Verpoorte, 2008). The highest content of *N-trans*-caffeoyltyramine was recorded in Beldia Jebha (721 µg g^{-1}), followed by Beldia Galaz (680 µg g^{-1}) and Critical Jebha (541 µg g^{-1}). However, Tamorot and Ratba seeds showed lower values ranging from 447 µg g^{-1} for Critical Ratba to 390 µg g^{-1} for Critical Tamorot. Similarly, the seeds collected from Jebha and Galaz had high values of cannabisins A and B, unlike Ratba and Tamorot. Beldia Galaz displayed the highest values reaching 393 and 331 µg CTE g^{-1}, while Critical Tamorot had the lowest amounts, 217 and 195 µg CTE g^{-1}, for cannabisins A and B respectively (Table 2).

In a previous study comparing seven varieties of cannabis grown in Greece for 3 consecutive years, the authors recorded an *N-trans*-caffeoyltyramine content ranging from 14.8 to 83.2 mg 100 g^{-1} and a cannabisin A content, varying from 51.1 to 159.1 mg 100 g^{-1}, respectively, for Tygra and Felina cultivars (Irakli *et al.*, 2019). Likewise, *N-trans*-caffeoyltyramine in the bound phenolic fraction extracted from seeds of three Italian cannabis varieties fluctuated between 226.2 and 426.7 µg g^{-1} of seeds (Menga *et al.*, 2022). All these results were consistent with our findings. On the contrary, another recent study concerning the hemp variety CRS1 grown in Tasmania in 2019 reported a content of 128, 47.43, 10.59, 18.22 and 8.60 µg CTE g^{-1} for *N-trans*-caffeoyltyramine, feruloyltyramine, cannabisin A, cannabisin B and cannabisin C, respectively (Leonard *et al.*, 2021), which are lower than our results. In addition, a concentration of total phenylpropanoids (including 14 compounds) was found in hemp seeds from China, reaching 233.52 ± 2.50 µg mg^{-1} extract (Zhou *et al.*, 2018). Still, we cannot compare it with our values, given the different units used to express the results. Another study found values of 15.3–36.1 µg g^{-1} of caffeoyltyramine, 0.01–1.6 µg g^{-1} of cannabis A, 0.4–0.5 µg g^{-1} of cannabisin B and 0.02–0.14 µg g^{-1} of cannabisin C in the inflorescences of four cannabis cultivars. These values are lower than ours, which is more consistent with the literature proving that cannabis seeds are richer in phenylpropanoids than the other plant parts (Izzo *et al.*, 2020).

Table 3 represents the analysis of variance for the phenolic classes and the four major identified compounds, namely *N-trans*-caffeoyltyramine and cannabisins A, B and C. The ANOVA results showed that the contents of total phenolic acids, total lignanamides, total cannabinoids and total phenolic compounds were significantly ($P < 0.05$) affected by variety, geographic location and their interaction. However, the content of

Table 3 Analysis of variance for *N-trans*-caffeoyltyramine, cannabisins A, B and C and phenolic compound classes in the studied hemp seed populations

Source of variation	df	Mean squares	*F*-value	*P*-value
N-trans-caffeoyltyramine1				
Variety	1	65373.194	14.957	<0.0001
Growing area	3	220188.620	50.379	<0.0001
Variety × growing area	2	61724.520	14.123	<0.0001
Error	56	4370.622		
Cannabisin A				
Variety	1	2376.723	2.303	0.135
Growing area	3	48535.013	47.037	<0.0001
Variety × growing area	2	6024.868	5.839	0.005
Error	56	1031.84		
Cannabisin B				
Variety	1	284.787	0.491	0.486
Growing area	3	47505.770	81.875	<0.0001
Variety × growing area	2	8272.403	14.257	<0.0001
Error	56	580.223		
Cannabisin C				
Variety	1	290.464	3.391	0.071
Growing area	3	5143.927	60.050	<0.0001
Variety × growing area	2	821.912	9.595	<0.0001
Error	56	85.661		
Total phenolic acids				
Variety	1	23135.148	133.858	<0.0001
Growing area	3	18939.484	109.582	<0.0001
Variety × growing area	2	98.22	0.568	0.570
Error	56	172.834		
Total HCA				
Variety	1	44.227	0.007	0.936
Growing area	3	368844.154	54.717	<0.0001
Variety × growing area	2	218057.354	32.348	<0.0001
Error	56	6740.930		
Total lignanamides				
Variety	1	61691.760	9.550	0.003
Growing area	3	658826.174	101.989	<0.0001
Variety × growing area	2	162914.620	25.22	<0.0001
Error	56	6459.750		
Total phenylpropanoids				
Variety	1	65036.823	2.887	0.095
Growing area	3	1969122.319	87.398	<0.0001
Variety × growing area	2	757409.000	33.617	<0.0001
Error	56	22530.454		
Total Cannabinoids				
Variety	1	538.654	348.294	<0.0001
Growing area	3	5286.529	3418.278	<0.0001
Variety × growing area	2	13679.487	8845.176	<0.0001
Error	56	1.547		
Total phenolic compounds				
Variety	1	185179.603	7.704	0.007
Growing area	3	1920792.704	79.913	<0.0001
Variety × growing area	2	987603.869	41.089	<0.0001
Error	56	24035.904		

total HCA was significantly affected only by geographic location and its interaction with genotype. All the identified phenolic compounds were more affected by region than by genotype, which perfectly matches the results found in previous works concerning the total phenolic content in the whole seed extracts of different varieties of cannabis using the classical method of Folin–Ciocalteu (Taaifi *et al.*, 2021; Menga *et al.*, 2022).

According to the climatic data (Table S1) available for the cultivation year (2019), cumulative precipitation significantly influenced the content of phenolic compounds in cannabis seeds. The Ratba and Tamorot regions recorded the highest levels of rainfall (576 mm and 550 mm, respectively), followed by Galaz (470 mm) and Jebha (366 mm). Pearson's test showed a significant ($P < 0.05$) negative correlation between precipitation and total phenols ($r = -0.76$). In Galaz and Jebha, low-to-medium rainfall could induce moderate stress on cannabis plants, favouring the synthesis of phenolic compounds.

Irakli *et al.* (2019) found that the growing year (conditioned by the environment) significantly affected the phytochemicals and antioxidant activity of hemp seeds rather than genotypes, which corresponds perfectly to our results. Along with this, Faugno and his co-authors highlighted the effect of some agricultural practices, such as plant density and fertiliser use, on the phenol content of pressed hemp seed oil (Faugno *et al.*, 2019). Generally, the synthesis of phenolic compounds in cannabis and other plant species can be affected by several genetic and/or environmental factors and their interaction. Genetic factors reflect the genetic heritage of the variety, related to some genes favouring the production of secondary metabolites. Unlike other plant species, very few studies have been found in the literature concerning the genetic control of phenolic compound synthesis in cannabis (Bassolino *et al.*, 2020). Environmental factors are the external conditions of plant growth and development that influence the production of metabolites, such as temperature, light, water, altitude, soil and cultural practices. It is important to mention that the synthesis of phenolic compounds is favoured in plants when subjected to moderate biotic or abiotic stress since these compounds constitute a plant defence (Mansouri *et al.*, 2018). The genetic and environmental interaction also has a significant role in synthesising phenolic compounds because some environments affect gene expression.

Antioxidant activity of cannabis seeds

The seeds of two varieties of cannabis, grown in four regions of northern Morocco, were studied to assess their antioxidant power. Five tests were carried out: TAC, DPPH, ABTS, CUPRAC and FRAP, involving either the reducing power or the free radical scavenging ability. Table 4 lists the IC_{50} and EC_{50} values

Table 4 IC_{50} and EC_{50} values (Mean ± SD) for seed extracts from seven cannabis populations for TAC, DPPH, ABTS, CUPRAC and FRAP antioxidant tests (expressed in mg mL^{-1})

	Beldia				Critical				
Parameters	Jebha	Tamorot	Ratba	Galaz	Jebha	Tamorot	Ratba	Min	Max
TAC	1.83 ± 0.05a	2.98 ± 0.04c	3.59 ± 0.05e	3.12 ± 0.18d	2.62 ± 0.13b	3.69 ± 0.02f	4.14 ± 0.08g	1.75	4.26
DPPH	1.83 ± 0.05a	2.21 ± 0.03b	4.28 ± 0.31d	2.19 ± 0.06b	1.64 ± 0.00a	2.82 ± 0.14c	4.37 ± 0.18d	1.63	4.71
ABTS	2.45 ± 0.06a	3.10 ± 0.19b	5.62 ± 0.22d	3.21 ± 0.10b	2.45 ± 0.03a	4.07 ± 0.12c	6.02 ± 0.13e	2.34	6.21
CUPRAC	2.65 ± 0.02a	4.16 ± 0.11c	7.64 ± 0.27e	3.38 ± 0.13b	2.79 ± 0.12a	5.21 ± 0.11d	9.29 ± 0.25f	2.63	9.74
FRAP	2.38 ± 0.02c	2.70 ± 0.10d	4.37 ± 0.11g	2.20 ± 0.09b	1.75 ± 0.09a	2.91 ± 0.09e	3.69 ± 0.19f	1.60	4.59

Note: Different letters in the same line indicate significant differences ($p < 0.05$) between samples based on Tukey test.

Abbreviations: ABTS and DPPH radical scavenging activity; CUPRAC, cupric reducing antioxidant capacity; FRAP, Ferric reducing antioxidant power; TAC, Total antioxidant capacity.

recorded for the antioxidant tests for the studied cannabis populations. The seeds of the seven populations showed significant variability for all the tests. The IC_{50} and EC_{50} values were 1.83–4.14, 1.64–4.37, 2.45–6.02, 2.65–9.29 and 1.75–4.37 mg mL^{-1} of extract for TAC, DPPH, ABTS, CUPRAC and FRAP respectively.

For TAC, DPPH, ABTS and CUPRAC tests, the Beldia variety from the Jebha region exhibited the smallest IC_{50} values and, therefore, the most potent antioxidant activity, while the Critical variety from the Ratba region showed the weakest activity. The FRAP test recorded the highest activity for the Critical from Jebha (1.75 mg mL^{-1}) and the lowest for the Beldia from Ratba (4.37 mg mL^{-1}). Averaged over varieties and antioxidant tests, we notice that the Jebha region is the most conducive to having a high level of antioxidants in cannabis, followed by Galaz, Tamorot and Ratba.

Several studies have been interested in evaluating the antioxidant activity of different organs of cannabis. Regarding hemp seeds, most *in vitro* tests in the literature concern the DPPH assay and, to a minor extent, ABTS and FRAP tests. In a recent study, the DPPH-IC_{50} values for whole cannabis seeds from eight cultivars grown in Spain ranged from 2.5 to 9.2 mg mL^{-1} (Alonso-Esteban *et al.*, 2022). Also, Pojić *et al.* (2014) reported an IC_{50} value of 8 mg mL^{-1} for the hemp seed cake from the Helena variety using the DPPH test, which indicates a low antioxidant activity compared to all the varieties tested in our study.

On the other hand, a previous work studying separately defatted kernels and hulls showed IC_{50} values ranging from 0.10 to 1.32 mg mL^{-1} for DPPH and from 0.013 to 0.114 mg mL^{-1} for ABTS, reflecting an interesting antioxidant activity (Chen *et al.*, 2012). Furthermore, other studies have reported even lower IC_{50} in DPPH (0.2–0.5 mg mL^{-1}) and ABTS (0.03–0.5 mg mL^{-1}) of several hemp seed fractions (Rea Martinez *et al.*, 2020). Values of 69–91% DPPH inhibition and 17.39–32.53 μmol Fe^{2+} g^{-1} seeds for the FRAP test were obtained in seeds of seven Chinese varieties (Ning *et al.*, 2022). Despite the use of different methods or units to express their results, all these studies mentioned above prove the potential of cannabis seeds as a natural source of antioxidants.

The analysis of variance showed that the observed variations in antioxidant activities among samples were ascribed to the variety, growing area and their interaction ($P < 0.05$). It is noteworthy that the geographical area effect showed a large magnitude compared to the varietal effect (Table 5). Indeed, several studies have pointed out that the antioxidant power of several plant extracts varies according to the varieties, the seasons, the environmental conditions and the techniques of extraction and analysis of samples (Chrysargyris *et al.*, 2021; Bibi *et al.*, 2022). Interestingly, Menga *et al.* (2022) studied hemp seeds of three Italian cultivars for 2 consecutive years. They showed that 43.3% of the total variance of ABTS antioxidant activity was explained by the effect of the growth year (characterised by different climatic conditions) against only 11.6% for the genotype effect, which is consistent with our results.

Several studies have linked the antioxidant activity of extracts from several plant species, including cannabis seeds, to the content of secondary metabolites and, more particularly, phenolic compounds (Chen *et al.*, 2012; Ning *et al.*, 2022). Due to their structure, these substances can neutralise free radicals, chelate metals and/or reduce oxidants.

Chemometric analysis

Principal component analysis (PCA) was used to reduce the number of variables and assess the similarity level between hemp seeds produced in northern Morocco. The Kaiser–Meyer–Olkin (KMO) test was performed for all parameters to measure their sampling adequacy and suitability for factor analysis. Variables with a KMO index below 0.5 were

Table 5 Analysis of variance for antioxidant tests in the studied hemp seed populations

Source of variation	df	Mean squares	*F*-value	*P*-value
TAC				
Variety	1	6.263	695.961	<0.0001
Growing area	3	8.606	956.370	<0.0001
Variety × growing area	2	0.07	7.792	0.001
Error	56	0.009		
DPPH				
Variety	1	0.380	16.792	<0.0001
Growing area	3	21.933	969.188	<0.0001
Variety × growing area	2	0.745	32.94	<0.0001
Error	56	0.023		
ABTS				
Variety	1	2.774	152.115	<0.0001
Growing area	3	35.804	1963.081	<0.0001
Variety × growing area	2	1.064	58.319	<0.0001
Error	56	0.018		
CUPRAC				
Variety	1	12.174	441.718	<0.0001
Growing area	3	106.874	3877.695	<0.0001
Variety × growing area	2	2.598	94.25	<0.0001
Error	56	0.028		
FRAP				
Variety	1	1.826	150.649	<0.0001
Growing area	3	13.847	1142.390	<0.0001
Variety × growing area	2	1.130	93.194	<0.0001
Error	56	0.012		

Abbreviations: ABTS and DPPH radical scavenging activity; CUPRAC, cupric reducing antioxidant capacity; FRAP, Ferric reducing antioxidant power; TAC, Total antioxidant capacity.

eliminated (cannabisin D and cannabisin F). The final dataset consists of a 63 × 40 matrix, with 63 cannabis individuals (21 samples × 3 experimental replicates) and 40 variables. The PCA score plot of the studied cannabis samples is presented in Figs 1 and 2. The first component explains 44.15% of the total inertia, while the second explains 15.07%, which makes up 59.22%.

The variables contributing to the two principal components and their linear correlation coefficients are presented in Table S3 (Supplementary data). The first principal component (PC1) positively correlates with 24 variables comprising most of the identified phenolic compounds (21 compounds + total phenols, total HCA and total lignanamides). It is negatively correlated with the IC_{50} indices of antioxidant activity tests. On the other hand, the second principal component (PC2) is positively correlated with 3,3′-demethylheliotropamide and tri-*p*-coumaroylspermidine, but negatively correlated with total phenolic acids, *p*-coumaric acid and total cannabinoids (Supplementary Table S3).

According to the growing area, the PCA results showed well-delimited and differentiated groups except for Jebha and Galaz, which are superimposed (Fig. 1). According to the first axis (PC1), we can differentiate the Jebha and Galaz regions from the Ratba and Tamorot regions. Jebha and Galaz, showing high coordinates on PC1, were characterised by cannabis seeds having high contents of phenolic compounds and low values of IC_{50} and EC_{50}. On the other hand, Ratba and Tamorot, showing low coordinates on PC1, were characterised by low contents of phenolic compounds and high IC_{50} and EC_{50} values. The second axis (PC2) opposes the Ratba region to the Tamorot region. Ratba's cannabis seeds were characterised by a low amount of total phenolic acids, benzoic acid, coumaric acid and total cannabinoids and a high amount of tri-*p*-coumaroylspermidine and 3,3′-Demethylheliotropamide, unlike seeds from the Tamorot region.

According to the variety, the PCA results showed that the Critical and Beldia varieties overlap without a clear separation, which confirms that the varietal factor does not contribute as much as the regional factor in explaining the variations observed for the phenolic compounds and antioxidant activities. However, it can be observed that Critical variety constitutes a more homogeneous group than Beldia. In fact, the latter has more scattered individuals on the two-dimensional plane PC1 × PC2 (Fig. 2). This could be explained by the fact that Critical is a uniform hybrid variety resulting from guided crosses, unlike Beldia, which is a local ecotype with many phenotypic or even genotypic variations (biotypes). The PCA results showed that Critical variety has low coordinates on PC1, meaning it has low levels of phenolic compounds and high IC_{50} and EC_{50} values. In contrast, Beldia represents a considerable variation, with some individuals displaying very high coordinates, reflecting high levels of phenolic compounds and low IC_{50} and EC_{50} values.

A heatmap with implemented dendrograms was generated to summarise the results in a compact and easily readable way (Fig. 3). This exploratory method facilitates examining the relationships between all dataset points by juxtaposing the most similar samples. The rows are the cannabis populations, while the columns are the IC_{50} and EC_{50} values and the concentrations of the identified phenolic compounds. Each cell in the heatmap visualises the correlation between a studied variable (phenolic compounds and antioxidant activities) and a cannabis sample. The colour varies from red, representing high values, to blue, indicating low ones. The heatmap displays the variations in the identified phenolic compounds and antioxidant potency among different cannabis seed samples. As shown by the analysis of variance, these variations could be due to the climatic conditions of each region.

As shown in Fig. 3, two clusters of cannabis populations can be differentiated (at a distance of 11.06). The first cluster includes Beldia Jebha and Beldia Galaz, while the second contains the remaining five

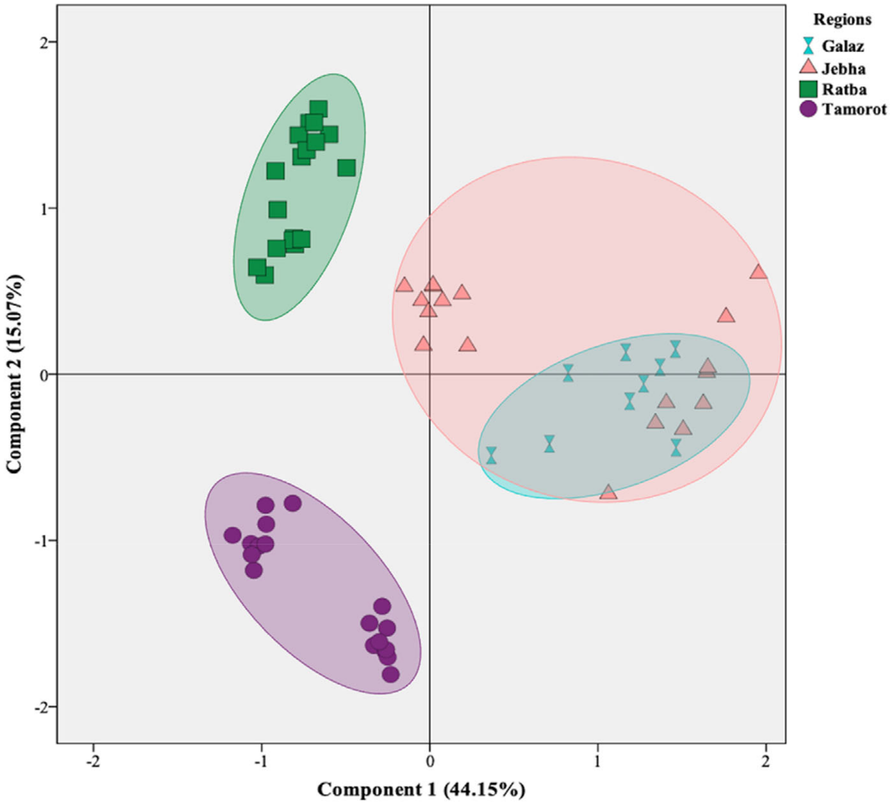

Figure 1 Principal component analysis score plot of the studied non-industrial hemp seeds separated according to their collection site in northern Morocco.

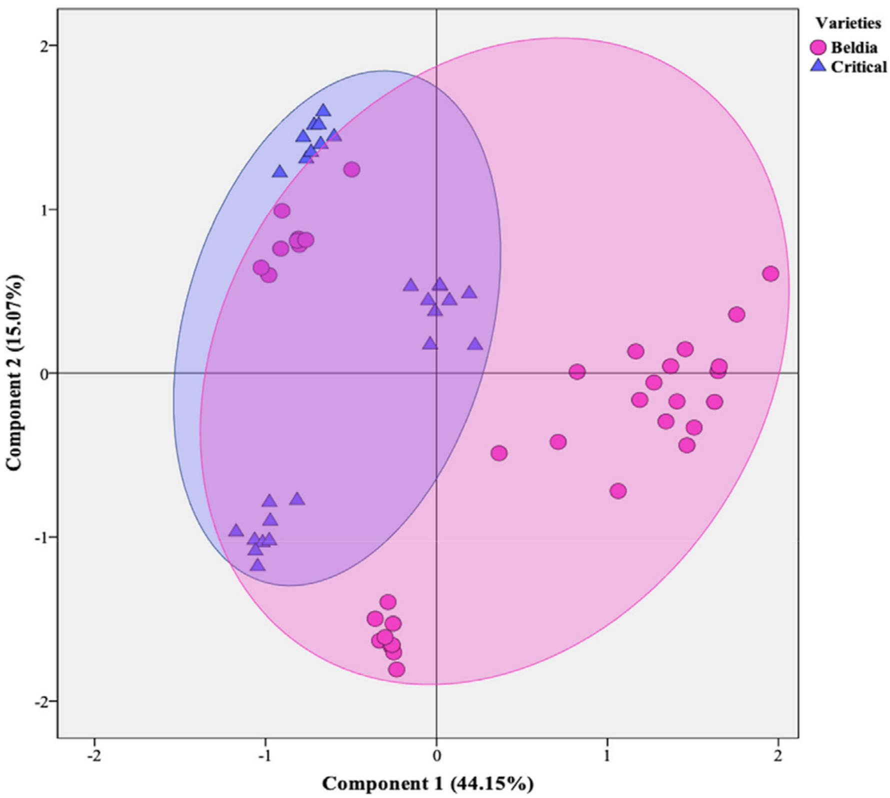

Figure 2 Principal component analysis score plot of the two studied varieties (Beldia and Critical) of non-industrial hemp seeds from four regions in northern Morocco.

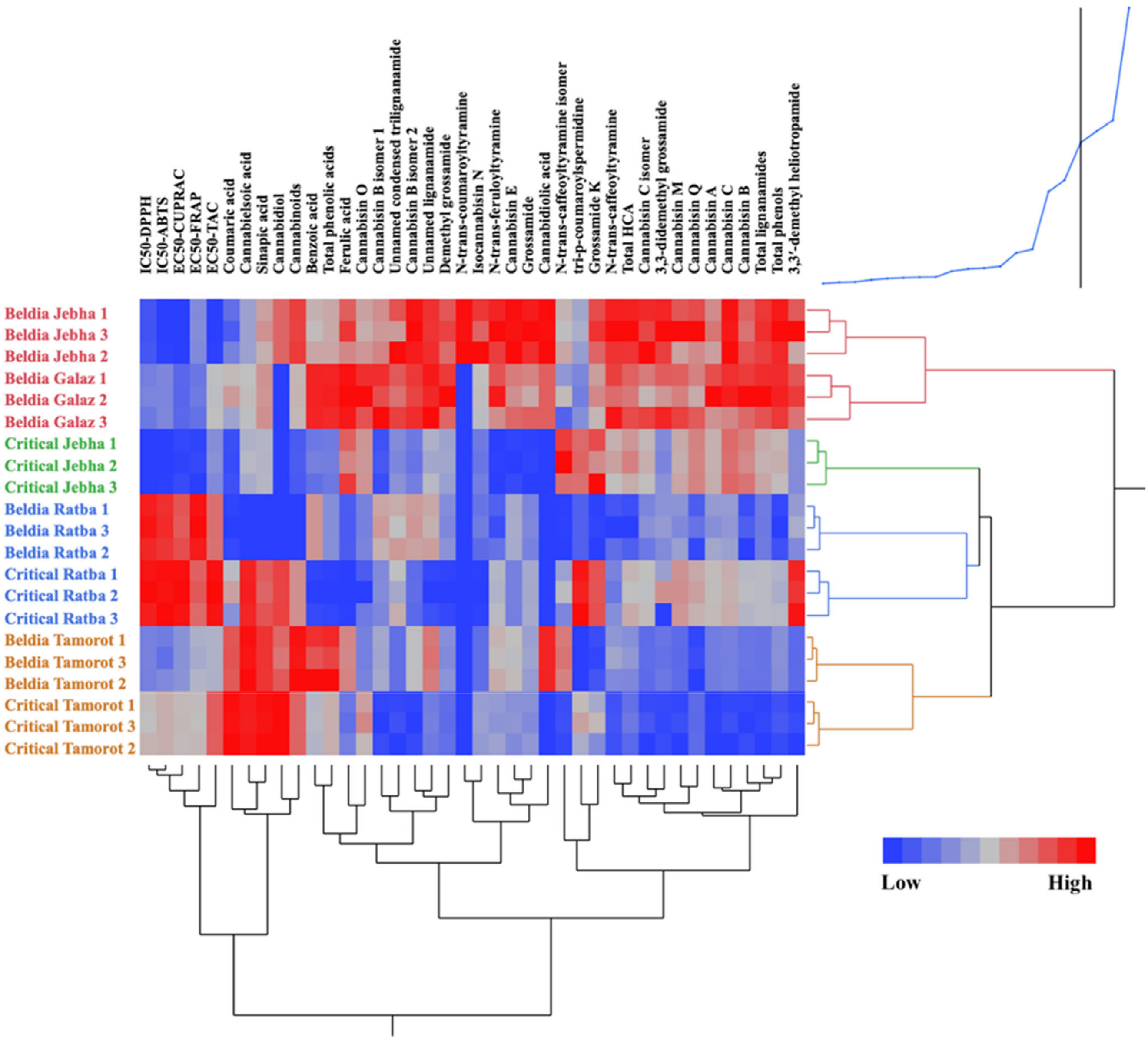

Figure 3 Cluster heatmap of the investigated Cannabis populations and the studied variables. Columns and rows represent the studied variables (phenolics compounds, IC_{50} and EC_{50} values) and Cannabis populations respectively. The clustering trees were constructed based on the Euclidean distance coefficient.

populations with a clear regional separation. These results are consistent with those from the PCA analysis. Our study also highlighted the importance of genetic and environmental interaction. This interaction allows judging the stability of each variety's behaviour and evaluating its adaptation to different environmental conditions. The Beldia variety, as a local ecotype, is more adapted to the Moroccan environment, thus ensuring higher levels of phenolic compounds, except for the Ratba region, where we notice a superiority of Critical. This could be explained by the climatic conditions experienced during this year, which somehow were more favourable to the Critical variety in this region.

A previous work from our research group, aiming to characterise the same cannabis populations in terms of fatty acids, triacylglycerols and tocopherols composition, found that these populations were discriminated mainly by the effect of variety. Critical was characterised by higher contents of polyunsaturated fatty acids (particularly C18:2n6) and trilinolein (LLL), with a richness in tocopherols (γ-tocopherol). In contrast, Beldia was characterised by its high contents of monounsaturated (C16:1, C18:1 and C20:1) and saturated fatty acids (C16:0, C20:0 and C24:0) with high oil content (Taaifi *et al.*, 2021). Combining these conclusions with the present study will better characterise these varieties grown in Morocco.

Conclusions

Characterisation of phenolic compounds in hemp seeds from two varieties grown in four Moroccan regions was performed using HPLC-DAD/ESI-MS^2 analysis. The hemp seed samples showed a rich phenolic profile comprising four phenolic classes: phenolic acids, hydroxycinnamic acid amides, lignanamides and cannabinoids. The relative content of each phenolic compound varied significantly among cannabis populations. The observed variability was mainly due to the geographical location and its interaction with the variety factor. These findings contribute to the better characterisation of cannabis seeds, which is crucial to their valorisation in industrial fields.

Acknowledgments

This research was supported by: the Moroccan Ministry of Higher Education, Scientific Research and innovation and the National Agency of Medicinal and Aromatic Plants through the VPMA2/ref 2020/1 project; the Wallonia Brussels International (WBI).

Author contributions

Chaymae Benkirane: Conceptualization (equal); formal analysis (lead); software (equal); writing – original draft (equal). **Farid Mansouri:** Conceptualization (equal); methodology (lead); software (equal); supervision (equal); writing – original draft (equal). **abdessamad ben moumen:** Formal analysis (equal); validation (equal). **Yassine Taaifi:** Data curation (equal); validation (equal). **Reda Melhaoui:** Data curation (equal); investigation (equal). **Hana Serghini Caid:** Validation (equal); visualization (equal); writing – review and editing (equal). **Marie Laure Fauconnier:** Project administration (equal); visualization (equal); writing – review and editing (equal). **Ahmed Elamrani:** Funding acquisition (lead); project administration (equal); visualization (equal). **Malika Abid:** Investigation (equal); supervision (equal); validation (equal).

Conflict of interest

The authors declare no conflict of interest associated with this work.

Ethics statement

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.16298.

Data availability statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

References

Alonso-Esteban, J.I., Pinela, J., Ćirić, A. *et al.* (2022). Chemical composition and biological activities of whole and dehulled hemp (*Cannabis sativa* L.) seeds. *Food Chemistry*, **374**, 1–9.

Bassolino, L., Buti, M., Fulvio, F. *et al.* (2020). In silico identification of myb and bhlh families reveals candidate transcription factors for secondary metabolic pathways in *Cannabis sativa* L. *Plants*, **9**, 1–19.

Benkirane, C., Moumen, A.B., Fauconnier, M.-L. *et al.* (2022). Bioactive compounds from hemp (*Cannabis sativa* L.) seeds: optimization of phenolic antioxidant extraction using simplex lattice mixture design and HPLC-DAD/ESI-MS^2 analysis. *RSC Advances*, **12**, 25764–25777.

Bibi, N., Shah, M.H., Khan, N. *et al.* (2022). Variations in total phenolic, total flavonoid contents, and free radicals' scavenging potential of onion varieties planted under diverse environmental conditions. *Plants*, **11**, 950.

Cattaneo, C., Givonetti, A., Leoni, V. *et al.* (2021). Biochemical aspects of seeds from *Cannabis sativa* L. plants grown in a mountain environment. *Scientific Reports*, **11**, 3927.

Chen, T., He, J., Zhang, J. *et al.* (2012). The isolation and identification of two compounds with predominant radical scavenging activity in hempseed (seed of *Cannabis sativa* L.). *Food Chemistry*, **134**, 1030–1037.

Chouvy, P.A. & Afsahi, K. (2014). Hashish revival in Morocco. *International Journal of Drug Policy*, **25**, 416–423.

Chrysargyris, A., Evangelides, E. & Tzortzakis, N. (2021). Seasonal variation of antioxidant capacity, phenols, minerals and essential oil components of sage, spearmint and Sideritis plants grown at different altitudes. *Agronomy*, **11**, 1766.

Crini, G., Lichtfouse, E., Chanet, G. & Morin-Crini, N. (2020). Applications of hemp in textiles, paper industry, insulation and building materials, horticulture, animal nutrition, food and beverages, nutraceuticals, cosmetics and hygiene, medicine, agrochemistry, energy production and environment: a review. *Environmental Chemistry Letters*, **18**, 1451–1476.

Farinon, B., Molinari, R., Costantini, L. & Merendino, N. (2020). The seed of industrial hemp (*Cannabis sativa* L.): nutritional quality and potential functionality for human health and nutrition. *Nutrients*, **12**, 1935.

Faugno, S., Piccolella, S., Sannino, M. *et al.* (2019). Can agronomic practices and cold-pressing extraction parameters affect phenols and polyphenols content in hempseed oils? *Industrial Crops and Products*, **130**, 511–519.

Flores-Sanchez, I.J. & Verpoorte, R. (2008). Secondary metabolism in cannabis. *Phytochemistry Reviews*, **7**, 615–639.

Frassinetti, S., Moccia, E., Caltavuturo, L. *et al.* (2018). Nutraceutical potential of hemp (*Cannabis sativa* L.) seeds and sprouts. *Food Chemistry*, **262**, 56–66.

Glivar, T., Er, J., Kreft, S. *et al.* (2020). Cannabinoid content in industrial hemp (*Cannabis sativa* L.) varieties grown in Slovenia. *Industrial Crops and Products*, **145**, 112028.

Grochowski, D.M., Uysal, S., Aktumsek, A. *et al.* (2017). In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of *Potentilla thuringiaca*. *Phytochemistry Letters*, **20**, 365–372.

Huang, W., Cai, Y., Zhang, Y., Huang, W. & Cai, Y. (2009). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and Cancer*, **62**, 1–20.

Irakli, M., Tsaliki, E., Kalivas, A., Kleisiaris, F., Sarrou, E. & Cook, C.M. (2019). Effect of genotype and growing year on the nutritional, phytochemical, and antioxidant properties of industrial hemp (*Cannabis sativa* L.) seeds. *Antioxidants*, **8**, 491.

The authors of this reference studied and explained the influence of the genotype and growing year conditions on the phytochemical composition and antioxidant activity of industrial cannabis seeds. Their results consolidate our findings.

Isidore, E., Karim, H. & Ioannou, I. (2021). Extraction of phenolic compounds and terpenes from *Cannabis sativa* L. by-products: from conventional to intensified processes. *Antioxidants*, **10**, 942.

Izzo, L., Castaldo, L., Narváez, A. *et al.* (2020). Analysis of phenolic compounds in commercial *Cannabis sativa* L. inflorescences using UHPLC-Q-orbitrap HRMS. *Molecules*, **25**, 631.

Kadriye, I., Demirata, B. & Apak, R. (2012). Determination of total antioxidant capacity of lipophilic and hydrophilic antioxidants in the same solution by using ferric – ferricyanide assay. *Food Analytical Methods*, **5**, 1150–1158.

Leonard, W., Zhang, P., Ying, D. & Fang, Z. (2020a). Hempseed in food industry: nutritional value, health benefits, and industrial applications. *Comprehensive Reviews in Food Science and Food Safety*, **19**, 282–308.

Leonard, W., Zhang, P., Ying, D. & Fang, Z. (2020b). Lignanamides: sources, biosynthesis and potential health benefits–a minireview. *Critical Reviews in Food Science and Nutrition*, **61**, 1404–1414.

Leonard, W., Zhang, P., Ying, D., Xiong, Y. & Fang, Z. (2021). Extrusion improves the phenolic profile and biological activities of hempseed (*Cannabis sativa* L.) hull. *Food Chemistry*, **346**, 128606.

This reference reported the contents of some phenolamides in cannabis seeds. It was used to compare our phenolic compounds' contents and, therefore, to indicate our results' validity.

Mansouri, F., Moumen, A.B., Belhaj, K. *et al.* (2018). Effect of crop season on the quality and composition of extra virgin olive oils from Greek and Spanish varieties grown in the oriental region of Morocco. *Emirates Journal of Food and Agriculture*, **30**, 549–562.

Menga, V., Garofalo, C., Suriano, S. *et al.* (2022). Phenolic acid composition and antioxidant activity of whole and defatted seeds of Italian hemp cultivars: a two-year case study. *Agriculture (Switzerland)*, **12**, 759.

Moccia, S., Siano, F., Russo, G.L. *et al.* (2020). Antiproliferative and antioxidant effect of polar hemp extracts (*Cannabis sativa* L., fedora cv.) in human colorectal cell lines. *International Journal of Food Sciences and Nutrition*, **71**, 410–423.

Mutha, R.E., Tatiya, A.U. & Surana, S.J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future Journal of Pharmaceutical Sciences*, **7**, 25.

Nigro, E., Crescente, G., Formato, M. *et al.* (2020). Hempseed lignanamides rich-fraction: chemical investigation and cytotoxicity towards U-87 glioblastoma cells. *Molecules*, **25**, 1–25.

This reference constitutes an important database for the mass spectra of hydroxycinnamic acid amides, lignanamides and flavonoids contained in cannabis seeds. It was used to identify phenolic compounds in our hemp seed extracts by comparing the spectral data of each compound.

Ning, K., Hou, C., Wei, X. *et al.* (2022). Metabolomics analysis revealed the characteristic metabolites of hemp seeds varieties and metabolites responsible for antioxidant properties. *Frontiers in Plant Science*, **13**, 904163.

Pojić, M., Mišan, A., Sakač, M. *et al.* (2014). Characterization of byproducts originating from hemp oil processing. *Journal of Agricultural and Food Chemistry*, **62**, 12436–12442.

Pollastro, F., Minassi, A. & Fresu, L.G. (2018). Cannabis phenolics and their bioactivities. *Current Medicinal Chemistry*, **25**, 1160–1185.

Rea Martinez, J., Montserrat-De La Paz, S., De La Puerta, R., García-Giménez, M.D. & Fernández-Arche, M.Á. (2020). Characterization of bioactive compounds in defatted hempseed (*Cannabis sativa* L.) by UHPLC-HRMS/MS and anti-inflammatory activity in primary human monocytes. *Food and Function*, **11**, 4057–4066.

Roumani, M., Duval, R.E., Ropars, A., Risler, A., Robin, C. & Larbat, R. (2020). Phenolamides: plant specialized metabolites with a wide range of promising pharmacological and health-promoting interests. *Biomedicine and Pharmacotherapy*, **131**, 110762.

This review discusses the structure and various biological activities of phenolamides, the most abundant phenolic compounds in cannabis seeds.

Schluttenhofer, C. & Yuan, L. (2017). Challenges towards revitalizing hemp: a multifaceted crop. *Trends in Plant Science*, **22**, 917–929.

Taaifi, Y., Benmoumen, A., Belhaj, K. *et al.* (2021). Seed composition of non-industrial hemp (*Cannabis sativa* L.) varieties from four regions in northern Morocco. *International Journal of Food Science & Technology*, **56**, 5931–5947.

This work is of great importance since it studied the seeds of non-industrial cannabis grown in Morocco. The authors investigated the effect of genotype and environment on the fatty acid, triacylglycerol and tocopherol composition. It provides, along with our results, a better characterization of cannabis seeds grown in Morocco.

Zhou, Y., Wang, S., Ji, J., Lou, H. & Fan, P. (2018). Hemp (*Cannabis sativa* L.) seed Phenylpropionamides composition and effects on memory dysfunction and biomarkers of neuroinflammation induced by lipopolysaccharide in mice. *ACS Omega*, **3**, 15988–15995.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 HPLC chromatogram (at 280 nm) for the extracted phenolic compounds from hemp seeds in the Beldia variety.

Fig. S2 MS2 spectra and UV–visible spectrum of the identified phenolic compounds extracted from Moroccan hemp seeds.

Table S1 The geographical coordinates, altitude, and climatic conditions during 2019 of four Moroccan regions of cannabis cultivation

Table S2 Chromatographic and MS^2 spectral data for the identified phenolic compounds in cannabis seeds in positive mode $[M + H]^+$

Table S3 Component matrix after principal component analysis of the phenolic compound composition of two hemp (*Cannabis sativa* L.) varieties from four regions in northern Morocco