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Cosmetic potential of phenolic-rich extracts from Cannabis sativa L. seeds: Inhibition of enzymes involved in skin aging, inflammation and hyperpigmentation



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**Cosmetic potential of phenolic-rich extracts from *Cannabis sativa* L. seeds:
Inhibition of enzymes involved in skin aging, inflammation and
hyperpigmentation**

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Abstract

The demand for natural and multifunctional ingredients in cosmetic formulations has surged in recent years, driven by growing consumer awareness of skin health. In this context, the present study investigates the cosmetic potential of *Cannabis sativa* L. seeds. Two fractions, obtained by solvent partitioning with petroleum ether and ethyl acetate, were assessed for their radical scavenging ability (DPPH, ABTS, HRSA) and reducing power (FRAP, CUPRAC, TAC). The ethyl acetate (EA) fraction exhibited significantly higher antioxidant activity and was therefore selected for further evaluation of its inhibitory effects on key enzymes associated with skin aging, inflammation, and hyperpigmentation. The phenolic compounds in the EA fraction were analyzed by HPLC-DAD coupled with mass spectrometry in negative ion mode using an untargeted approach. The EA fraction demonstrated strong enzyme-inhibitory activities ($IC_{50} = 31.05 \mu\text{g mL}^{-1}$, $75.53 \mu\text{g mL}^{-1}$, $5.71 \mu\text{g mL}^{-1}$, and $141.72 \mu\text{g mL}^{-1}$, respectively for elastase, collagenase, lipoxygenase, and tyrosinase), likely due to its high contents of hydroxycinnamic acid amides (240.90 mg g^{-1} extract, predominantly represented by caffeoyltyramine) and lignanamides ($449.34 \text{ mg CTE g}^{-1}$ extract, dominated by cannabisisins A and B). These findings indicate that phenolic-rich fractions from cannabis seeds represent promising natural ingredients for cosmetic formulations targeting oxidative stress, skin aging, hyperpigmentation, and cutaneous inflammation. The results further contribute to the valorization of cannabis seed by-products and support the development of safer, plant-based alternatives in the cosmetic industry.

Keywords: *Cannabis sativa* L., seeds, solvent fractionation, cosmetics, antioxidant activity, enzyme inhibition.

1- Introduction

The cosmetic industry plays a vital role in modern society, contributing to personal well-being, while representing a rapidly growing market with substantial economic impact. Among the most prominent concerns in this sector are skin aging, inflammation-related skin disorders, and hyperpigmentation. These conditions markedly affect skin appearance and are often linked to environmental stressors (*e.g.* ultraviolet radiation), intrinsic aging processes, and biological imbalances such as hormonal disorders and oxidative stress [1–3].

Skin aging is marked by reduced elasticity and the appearance of wrinkles and sagging, largely due to decreased synthesis of collagen and elastin, the main structural components of the skin's extracellular matrix [4]. This process is accelerated by matrix metalloproteinases such as collagenase and elastase, which degrade collagen and elastin, respectively; their inhibition is therefore a central strategy in anti-aging skincare [5]. In contrast, cutaneous inflammation arises from the activation of immune pathways and the overproduction of inflammatory mediators, that disrupt normal skin homeostasis [6]. Lipoxygenases (LOX) play a key role in this process by generating pro-inflammatory lipid mediators, including leukotrienes and hydroxyeicosatetraenoic acids [7]. The overproduction of these metabolites amplifies inflammatory signaling and contributes to several inflammation-related skin disorders, including acne, eczema, and photo-induced irritation. Consequently, lipoxygenase inhibition is a relevant approach for soothing and anti-inflammatory cosmetic applications. Hyperpigmentation refers to the darkening of specific areas of the skin due to an overproduction and accumulation of melanin, with tyrosinase acting as the rate-limiting enzyme in melanogenesis [8]. Inhibiting tyrosinase reduces melanin synthesis and is therefore a well-established strategy for managing pigmentary disorders like melasma and solar lentigos (age spots), and promoting an even skin tone [9].

Overall, the inhibition of elastase, collagenase, lipoxygenase, and tyrosinase plays a central role in maintaining skin health. However, active ingredients, commonly used for these purposes, have raised concerns due to potential health risks, including toxicity, instability, or long-term side effects. These issues have led to regulatory restrictions in some regions and have fueled growing interest in identifying safer, more effective, and stable alternatives. As a result, natural compounds are increasingly being explored for their potential in skin care, particularly for applications such as skin lightening and anti-aging [3,10].

Cannabis (*Cannabis sativa* L.) seeds, botanically classified as achenes (dry indehiscent fruits enclosing the true seed), are often overlooked compared to the other parts of the plant [11]. Nevertheless, they represent a rich and sustainable source of several bioactive compounds, including phenolic acids, flavonoids, and particularly phenylpropanoid amides [12,13]. The use of these plant-derived phenolic compounds has gained increasing attention due to their antioxidant, anti-inflammatory, and skin-protecting properties, offering strong potential for the development of natural and innovative cosmetic products.

The use of cannabis seeds for cosmetic purposes has primarily focused on the extraction and application of their oil. Cannabis seed oil has long been valued for its strengthening, moisturizing, and softening effects on both hair and skin, as recognized in traditional cultural practices across various regions, including Morocco [14]. In recent years, cannabis seed oil has also gained popularity as an ingredient in numerous products, such as soaps, shampoos, and creams [15]. These benefits are largely attributed to its high content of essential fatty acids and tocopherols [16,17]. However, the remaining part of the seed, the pressed cake, remains underutilized despite its richness in phenolic compounds. This makes it an appealing target for further research, offering promising opportunities to enhance the value and application of cannabis seeds within the cosmetic industry.

Our study aims (i) to obtain a phenolic-rich extract from defatted cannabis seeds through solvent bio-guided fractionations, and (ii) to assess its antioxidant potential and inhibitory activities against elastase, collagenase, lipoxygenase, and tyrosinase enzymes. The results will provide scientific evidence supporting the use of these extracts as alternative active ingredients in cosmetic formulations targeting skin aging, hyperpigmentation disorders, and skin inflammation.

2- Materials and methods

2-1- Plant material and reagents

Seeds of *Beldia*, a local cannabis population commonly cultivated in northern Morocco, were kindly provided by the National Agency of Medicinal and Aromatic Plants (ANPMA), Taounate, Morocco. The cultivation of cannabis and the production of seeds were carried out in 2022 at the ANPMA experimental station. All reagents, standards, solvents, and enzymes, were purchased from Sigma Aldrich company.

2-2- Extraction and fractionation

Cannabis seeds (100 g) were prepared and extracted as previously described by Benkirane et al. [18] using 50% acetone-water mixture with a solid: liquid ratio of 1:10. The resulting extract was centrifuged and the acetone fraction was evaporated using a rotary evaporator at 40 °C. The aqueous resulting fraction was then fractionated successively using petroleum ether (100mL x3), ethyl acetate (100mL x3), and *n*-butanol (100mL x3). Each phase was separated, evaporated to dryness, and the resulting dry residues were reconstituted in methanol for subsequent analyses. Figure 1 illustrates the complete workflow of the extraction and fractionation procedures.

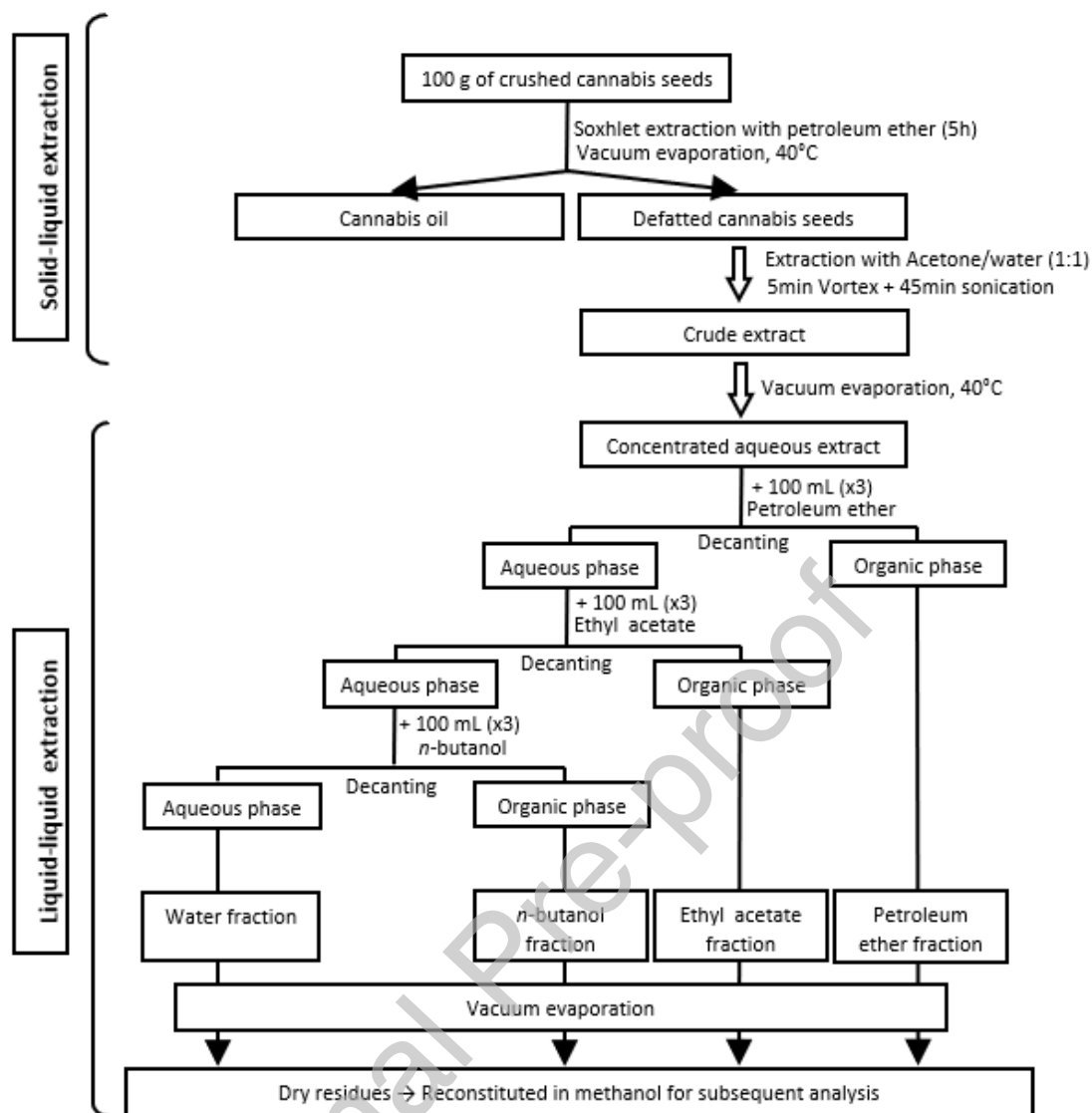


Figure 1: Flowchart of the extraction and fractionation procedures of *Cannabis* seeds.

2-3- Phenolic compound identification and semi-quantification

The phenolic profile of the obtained fractions was first qualitatively analyzed using high-performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD/ESI-MS²). Prior to injection, the methanol-dissolved fractions were filtered through a 0.22 µm membrane filter. Chromatographic separation and compound identification were performed as described previously by Benkirane et al. [19]. Briefly, separation was achieved on an Eclipse XDB-C18 column (3.5 µm, 150 × 4.6 mm; Agilent Technologies, USA) on an Agilent 1260 Infinity II HPLC system equipped with a diode array

detector (DAD). The mobile phase consisted of water (A) and acetonitrile (B), both containing 1% formic acid, with elution performed using a linear gradient from 2 to 100% B over 50 min. The injection volume was 10 μL and the flow rate was 0.6 mL min^{-1} . The profiles of HPLC-DAD were visualized and analyzed using Agilent OpenLAB CDS software.

After separation, individual peaks were collected and analyzed by mass spectrometry using an ion trap mass spectrometer (Esquire HCT, Bruker Daltonics, Germany) equipped with an electrospray ionization (ESI) source. Mass spectra were acquired in negative ionization modes by direct infusion (500 $\mu\text{L h}^{-1}$). The ESI parameters were set as follows: spray voltage 4500 V, dry gas temperature 200 $^{\circ}\text{C}$, nebulizer pressure 10 psi, and dry gas flow rate 4 L min^{-1} . MS^2 spectra were obtained by collision-induced dissociation with collision energies of 1–10%, over a mass range of 50–1000 m/z . Instrument calibration was performed using an ESI tuning mix. Instrument control and data acquisition were ensured via the Esquire Control software, while mass data processing was performed using the ACD/labs software. Phenolic compounds were identified by comparison of their MS, MS^2 fragmentation patterns, and UV spectra with published data.

Semiquantitative analysis was carried out for the ethyl acetate fraction using the HPLC-DAD profile acquired at 280 nm, as this wavelength corresponds to a common absorption maximum for the majority of the identified phenolic compounds. Due to the limited commercial availability of most identified lignanamides and hydroxycinnamic acid amides, their contents were semi-quantitatively estimated using an external calibration curve of *N-trans*-caffeoyltyramine (60-980 $\mu\text{g mL}^{-1}$; Limit of detection LOD = 34 $\mu\text{g mL}^{-1}$; Limit of quantification LOQ = 102 $\mu\text{g mL}^{-1}$). Results are therefore expressed as mg *N-trans*-caffeoyltyramine equivalents per g of extract (mg CTE g^{-1} extract). In contrast, benzoic acid (60-980 $\mu\text{g mL}^{-1}$; LOD = 48 $\mu\text{g mL}^{-1}$; LOQ = 146 $\mu\text{g mL}^{-1}$), *p*-coumaric acid (75-1200 μg

mL^{-1} ; LOD = $24 \mu\text{g mL}^{-1}$; LOQ = $73 \mu\text{g mL}^{-1}$), and ferulic acid ($60\text{-}980 \mu\text{g mL}^{-1}$; LOD = $34 \mu\text{g mL}^{-1}$; LOQ = $104 \mu\text{g mL}^{-1}$), were quantified using their respective commercial standards.

2-4- Antioxidant activity measurement

The antioxidant activity of the ethyl acetate and petroleum ether fractions was evaluated by different spectrophotometric tests using a UV-Visible spectrophotometer (Jenway 7305, France). The DPPH (DPPH• radical scavenging), ABTS (ABTS radical cation scavenging), FRAP (Ferric Reducing Antioxidant Power), CUPRAC (cupric reducing antioxidant capacity), and TAC (Total Antioxidant Capacity) tests were carried out as described by Benkirane et al. [19], with minor modifications. Specifically, all used extract and reagent volumes were proportionally adjusted for small-volume cuvettes (1mL).

Moreover, an additional antioxidant test was performed, namely Hydroxyl Radical Scavenging Activity (HRSA) following the protocol reported by Schmitt et al. [20], with some modifications. This activity was evaluated using the salicylate method, which relies on the ability of antioxidants to inhibit the formation of hydroxylated products of sodium salicylate. A volume of $400 \mu\text{L}$ of ferrous sulfate solution (1.5 mM) was mixed with $280 \mu\text{L}$ hydrogen peroxide (6 mM), and the mixture was incubated at 37°C for 45 minutes to allow the generation of hydroxyl radicals via the Fenton reaction. After incubation, $30 \mu\text{L}$ of the extract at different concentrations was added, followed by $120 \mu\text{L}$ of sodium salicylate solution (20 mM). The reaction mixture was further incubated at 37°C for 30 minutes. The absorbance was measured at 510 nm against a blank.

All antioxidant results were expressed as IC_{50} or EC_{50} values. IC_{50} (Median Inhibitory Concentration) is the concentration of extract that inhibits 50% of free radicals (used for the DPPH, ABTS, and HRSA). The EC_{50} (Median Effective Concentration) is the concentration that ensures 50% of the maximum response normalized by an absorbance of 1 (used for the

TAC, FRAP, and CUPRAC tests). The IC_{50} and EC_{50} values were calculated based on a dose-response model by the GraphPad Prism 9.4.0 software. Ascorbic acid was used as a positive control in all antioxidant assays under the same experimental conditions.

2-5- Enzyme inhibition assays

Four enzymes were involved in this study - elastase, collagenase, lipoxxygenase, and tyrosinase - to test their inhibition using the ethyl acetate fraction. All experiments were performed in triplicate. Test samples were evaluated at final concentrations ranging from 5 to 600 $\mu\text{g mL}^{-1}$. The enzymatic tests were spectrophotometrically performed and the absorbances were recorded for both the control and the sample. A_{control} is the absorbance of the control, reflecting the enzymatic activity (substrate + enzyme + buffer), while A_{sample} is the absorbance of the sample (substrate + enzyme + buffer + sample). It is noteworthy that both “control” and “sample” measurements were recorded using the corresponding blank, which contained all reagents except the enzyme. After calculating the inhibition percentages at various concentrations, the IC_{50} values (concentration required to inhibit 50% of the enzyme activity) were determined by non-linear regression analysis using GraphPad Prism 9.4.0 software.

2-5-1- Elastase inhibition

The inhibitory activity against elastase (derived from porcine pancreas) was evaluated based on the protocol reported by Dige et al. [21] with some modifications. The assay spectrophotometrically (BioTek Epoch microplate reader, Agilent) measures the release of *p*-nitroaniline, a chromogenic product generated by the enzymatic hydrolysis of the substrate N-succinyl-Ala-Ala-Ala-*p*-nitroanilide by elastase.

In each well of a 96-well microplate, 10 μL of the test sample was added to 80 μL of Tris-HCl buffer (0.2 M, pH 8.0) and 10 μL of elastase stock solution (0.5 U mL^{-1}). The plate

was pre-incubated at 25 °C for 10 minutes, after which 50 µL of N-succinyl-Ala-Ala-Ala-*p*-nitroanilide substrate (2mM) was added to initiate the reaction. The mixture was then incubated for 30 minutes at 25 °C, and the absorbance was recorded at 410 nm. Quercetin was used as positive control. The elastase inhibition activity (%) was calculated using the following equation:

$$\text{Elastase Inhibition (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad 1)$$

2-5-2- Collagenase inhibition assay

The collagenase inhibition assay was carried out as described by Roy et al. [2], with modifications. Collagenase from *Clostridium histolyticum* was used as well as its synthetic substrate N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), which mimics the natural structure of collagen. This assay was performed in a BioTek Epoch microplate (Agilent) spectrophotometer. The reaction was carried out in 50 mM Tricine buffer (pH 7.5), supplemented with 400 mM NaCl and 10 mM CaCl₂ to ensure optimal enzyme activity. A volume of 20 µL of the test sample was incubated with 20 µL of the collagenase enzyme (0.5mg mL⁻¹) and 40 µL of buffer for 15 minutes at 25°C. The enzymatic reaction was then initiated by the addition of 40 µL of the substrate (2mM). Absorbance was recorded at 335 nm immediately after substrate addition, and monitored continuously for 20-minute, to measure the decrease in absorbance, which correspond to the enzymatic cleavage of FALGPA by collagenase. Epigallocatechin-3-gallate (EGCG) was used as a positive control. The inhibition percentage was calculated based on the change in absorbance (ΔA) as follows:

$$\text{Collagenase inhibition (\%)} = \left(\frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \right) \times 100 \quad (2)$$

with $\Delta A = A_{t=0} - A_{t=20\text{min}}$

2-5-3- Lipoxygenase inhibition

The lipoxygenase (LOX) inhibitory activity of the extracts was evaluated spectrophotometrically using UV-visible spectrophotometer (Jenway 7305, France), according to the method of Andrade et al. [22] with modifications. The assay mixture contained 880 μ L of 0.1 M Tris-HCl buffer (pH 8.6), 10 μ L of soybean lipoxygenase solution (0.05mg/mL = 10000U/mL), and 20 μ L of the test sample. After pre-incubation at 25°C for 15 min, the reaction was initiated by adding 15 μ L of linoleic acid substrate (5 mM). The increase in absorbance was recorded at 234 nm for 5 min, corresponding to the formation of conjugated dienes. Quercetin was used as a positive control. The percentage of inhibition was calculated using the following equation:

$$\text{Lipoxygenase inhibition (\%)} = \left(\frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \right) \times 100 \quad (3)$$

with $\Delta A = A_{t=5\text{min}} - A_{t=0}$

2-5-4- Tyrosinase inhibition assay

The tyrosinase inhibition assay was performed in a microplate reader (BioTek Epoch, Agilent) using the protocol described by Grochowski et al. [23] with minor modifications. For this, we used tyrosinase (derived from mushroom) and L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate. Tyrosinase catalyzes the oxidation of L-DOPA into dopaquinone, an unstable intermediate that spontaneously undergoes cyclization and rearrangement to form dopachrome. This compound has a characteristic orange color, which is monitored spectrophotometrically. A volume of 25 μ L of the extract was added to 80 μ L of a tyrosinase solution (200 U mL⁻¹) and 100 μ L of phosphate buffer (40 mM, pH 6.8) in a 96-well microplate. The mixture was incubated for 15 minutes at 25 °C. The reaction was initiated by adding 40 μ L of a 10 mM solution of L-DOPA, followed by a 10-minute incubation at 25°C.

Absorbance was then measured at 492 nm. Kojic acid was used as positive control. Enzyme inhibition activity (%) was calculated using the following equation:

$$\text{Tyrosinase Inhibition (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad 4)$$

2-6- Statistical analysis

All results were presented as mean $\pm$ standard deviation (SD). Data were statistically analyzed using one way ANOVA analysis, followed by Tukey test to compare the antioxidant activities of both extracts (petroleum ether (PE) and ethyl acetate (EA)) and the positive control. Student test was also used to compare enzyme inhibition activities of EA fraction and their corresponding positive controls. The significance level was set at 5%. All statistical tests were generated and processed using IBM SPSS Statistics 25 software.

3- Results and discussion

In the present study, phenolic compounds were extracted from defatted cannabis seeds and subsequently concentrated through targeted fractionation. The resulting phenolic-rich fractions were then evaluated for their antioxidant activities. The most potent fraction was selected for further investigation of its cosmetic potential, specifically its ability to inhibit elastase, collagenase, lipoxygenase, and tyrosinase enzymes.

3-1- Phenolic screening of cannabis seed fractions

Following the extraction of defatted cannabis seeds, a sequential liquid-liquid fractionation was performed using petroleum ether (PE), ethyl acetate (EA), and *n*-butanol, yielding four distinct fractions: PE, EA, *n*-butanol, and an aqueous residue. This approach was designed to separate compounds based on their polarity, ranging from non-polar to highly polar constituents

The chromatographic profiles, obtained through preliminary HPLC profiling (Figure 2), clearly illustrate the chemical distribution across the four fractions. The PE fraction primarily contained the residues of apolar cannabinoids, as indicated by peaks at high retention times. Minor peaks corresponding to phenolic compounds were also detected, suggesting the presence of small amounts of some polar phenolics. In contrast, the EA fraction displayed numerous intense peaks throughout the chromatogram, particularly at intermediate retention times, reflecting a high content of moderately polar compounds, mainly phenolic compounds. This confirms the strong affinity of ethyl acetate, a semi-polar solvent, for extracting phenolics. The *n*-butanol and aqueous fractions, however, showed no significant peaks attributable to phenolic compounds. Instead, both chromatograms revealed strong signals at very early retention times, suggesting the presence of highly polar, water-soluble molecules, and likely including sugars, which do not absorb in the UV-Visible range.

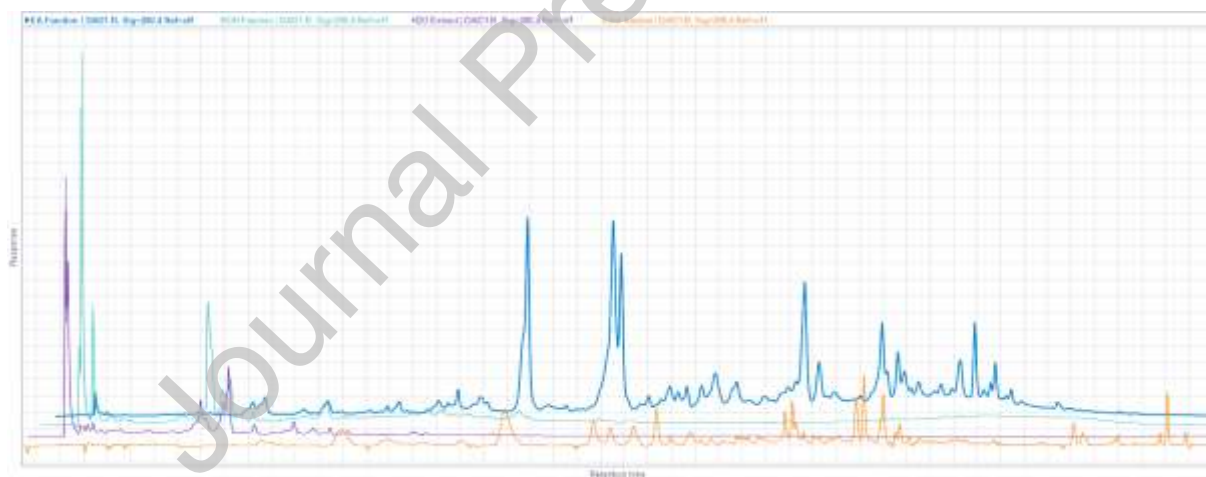


Figure 2: Chromatographic profiles of the obtained fractions monitored at 280 nm, showing ethyl acetate (blue), petroleum ether (orange), *n*-butanol (green), and aqueous (purple) fractions. All signals are equally scaled.

Overall, these results demonstrate the selectivity of the fractionation system: the EA fraction is enriched in phenolic compounds, the PE fraction contains lower amounts of

phenolics along with apolar compounds, and the *n*-butanol and aqueous fractions capture highly polar, non-phenolic constituents.

Our preliminary findings highlight the efficiency of the liquid-liquid extraction process in selectively enriching phenolic compounds in the EA fraction, consistent with previous studies. For instance, Rea Martinez et al. [13] reported that the ethyl acetate fraction obtained after 75% ethanol extraction was the richest in phenolics from cannabis seeds. Likewise, Kim et al. [24] demonstrated that sequential fractionation using *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol, yielded the most bioactive and phenolic-rich extract in the ethyl acetate fraction.

3-2- Antioxidant activities of PE and EA fractions from cannabis seeds

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense, is implicated in the pathogenesis of numerous chronic conditions and degenerative processes. In the context of skin health, this imbalance plays a pivotal role in premature aging and various dermatological issues. Elevated ROS levels can impair cellular function by damaging lipids, proteins, and DNA, thereby triggering inflammation, reducing collagen synthesis, and promoting hyperpigmentation [25,26]. Consequently, bioactive compounds with antioxidant properties are crucial for maintaining healthy skin. By neutralizing ROS and mitigating oxidative damage, these compounds preserve the skin's structural integrity and barrier function. Incorporating such antioxidants into cosmetic formulations can protect the skin from environmental stressors, delay the appearance of aging signs, and promote a more even and youthful complexion.

In this context, our study evaluated the antioxidant activity of two fractions derived from defatted cannabis seeds - petroleum ether (PE) and ethyl acetate fractions (EA) - using radical scavenging assays (DPPH, ABTS, HRSA) and redox-based assays (CUPRAC, FRAP,

TAC). The results are expressed as IC₅₀ or EC₅₀ values ($\mu\text{g mL}^{-1}$), with lower values indicating stronger activity (Table 1).

The ethyl acetate (EA) fraction exhibited the strongest radical scavenging activity with low IC₅₀ values for the DPPH ($9.36 \mu\text{g mL}^{-1}$) and ABTS ($8.07 \mu\text{g mL}^{-1}$) assays, comparable to the positive control (4.33 and $4.40 \mu\text{g mL}^{-1}$, respectively), and significantly outperforming the petroleum ether (PE) fraction (926.15 and $200.13 \mu\text{g mL}^{-1}$, respectively). In the hydroxyl radical scavenging assay, the EA fraction showed the best activity (IC₅₀= $39.21 \mu\text{g mL}^{-1}$) reinforcing its potent radical-quenching ability, followed by the PE fraction (IC₅₀= $46.53 \mu\text{g mL}^{-1}$), while ascorbic acid was less effective ($144.94 \mu\text{g mL}^{-1}$). These results suggest that the active compounds in EA fraction are highly effective against hydroxyl radicals, one of the most reactive and damaging types of ROS in biological systems.

Table 1: IC₅₀-EC₅₀ results of antioxidant activities of ethyl acetate and petroleum ether fractions from defatted cannabis seed extracts and their positive control.

	Ethyl acetate fraction	Petroleum ether fraction	Ascorbic acid
DPPH	9.36 ± 0.025 a	926.15 ± 5.74 b	4.33 ± 0.0026 a
ABTS	8.07 ± 0.19 a	200.13 ± 3.06 b	4.40 ± 0.01 a
CUPRAC	10.17 ± 0.04 a	113.29 ± 1.98 c	31.21 ± 2.28 b
FRAP	9.03 ± 0.69 b	91.78 ± 1.63 c	2.43 ± 0.2 a
TAC	28.33 ± 0.51 b	132.15 ± 3.6 c	12.09 ± 0.15 a
HRSA	39.21 ± 0.01 a	46.53 ± 0.4 b	144.94 ± 0.75 c

Different letters in each row mean statistically different ($p < 0.05$) based on Tukey test. All results were expressed in $\mu\text{g mL}^{-1}$

The ethyl acetate (EA) fraction also demonstrated higher antioxidant potential in the TAC ($28.33 \mu\text{g/mL}$) and FRAP ($9.03 \mu\text{g/mL}$) assays compared to the petroleum ether fraction (132.15 and $91.78 \mu\text{g/mL}$, respectively). However, ascorbic acid exhibited stronger activity than both fractions in FRAP ($2.43 \mu\text{g mL}^{-1}$) and TAC ($12.09 \mu\text{g mL}^{-1}$) assays. In CUPRAC

test, the EA fraction ($10.17 \mu\text{g mL}^{-1}$) outperformed both the PE fraction and ascorbic acid, further highlighting its potent antioxidant potential. Overall, these results clearly demonstrate that the EA fraction possesses the most promising and broad-spectrum antioxidant activity, likely due to its higher content of antioxidant phenolic compounds. Nevertheless, further investigations using cellular or *in vivo* models are warranted to confirm the biological relevance of these findings.

Comparatively, Chen et al. [27] reported lower potential in cannabis seed hull extracts from two Chinese varieties, with DPPH- IC_{50} and ABTS- IC_{50} values ranging from 0.09 to 2.21 mg mL^{-1} and 0.012-0.138 mg mL^{-1} , respectively, depending on the extraction solvent. Furthermore, all results obtained for the ethyl acetate fraction in the present study showed higher activity than those reported in our previous investigation using the same *Beldia* plant material [18]. In that study, the antioxidant activity ($\text{IC}_{50}/\text{EC}_{50}$) of *Beldia* samples from four different regions ranged between 1.83-3.59, 1.83-4.28, 2.45-5.62, 2.65-7.64, 2.83-4.37 mg mL^{-1} , for TAC, DPPH, ABTS, CUPRAC, and FRAP assays, respectively. Another study conducted on seeds from a Thai variety reported a DPPH- IC_{50} value of 14.39 mg mL^{-1} (Vs. $12 \mu\text{g mL}^{-1}$ for ascorbic acid), indicating lower antioxidant activity compared to our findings [28].

Although the seeds in these studies represented different varieties/populations from different regions (which likely explains a part of the observed variations), the fractionation process used in our study also played a key role by concentrating the phenolic compounds responsible for the enhanced antioxidant activity. In summary, these findings underscore the importance of solvent polarity in modulating the antioxidant profile of cannabis seed fractions.

The antioxidant activity of phenolic compounds is strongly influenced by their molecular structure. Several studies have specifically examined phenolic acid amides

(HCAAs) and their synthesized analogs to investigate the relationship between structural features and antioxidant capacity, particularly in terms of free radical scavenging activity. A consistent finding across these studies is the critical role of hydroxyl groups. For example, Marinova et al. [29] demonstrated that hydroxyl groups present in the amino moiety of molecules such as dopamine and tyramine derivatives contribute directly to the stabilization of free radicals. In contrast, amides lacking these hydroxyl groups tend to undergo side reactions that perpetuate oxidative processes. Notably, cannabis seeds are rich in HCAAs and related derivatives containing the tyramine moiety, further reinforcing their potential antioxidant activity [18,30].

Furthermore, Son and Lewis [31] demonstrated that radical scavenging activity increases with the number of hydroxyl or catechol moieties, as well as the presence of other hydrogen-donating functional groups such as -SH and -NH-CO. In this context, all HCAAs and their derivatives identified in cannabis seeds contain at least one -NH group, which may further contribute to their antioxidant properties. Complementing these findings, Kabanda [32] conducted a theoretical study, showing that phenolic -OH groups are more effective hydrogen donors than -NH groups, and that their para-position on the aromatic ring particularly enhances radical scavenging ability. The study also indicated that phenolic acid amides are more effective antioxidants than their parent aromatic amines or phenolic acids, due to their favorable thermodynamic profiles.

3-3- Phenolic profiling and enzyme inhibition activity of cannabis seed EA fraction

The results from all antioxidant assays reveal clear differences between the two cannabis seed fractions. The EA fraction consistently exhibited stronger activity than the PE fraction. This enhanced performance is likely attributed to its higher phenolic content, supporting the conclusion that fractionation was an effective strategy for concentrating bioactive compounds. Consequently, the EA fraction was selected for detailed quantitative

analysis to further clarify its phenolic profile. Moreover, this fraction was evaluated for its elastase, collagenase, lipoxygenase, and tyrosinase inhibition activities.

3-3-1- Phenolic profiling of the EA fraction using HPLC-DAD/ESI- MS²

HPLC-DAD/ESI-MS² analysis revealed a chemically rich profile in the ethyl acetate fraction of defatted *Cannabis sativa* seeds, dominated by hydroxycinnamic acid amides (HCAA) and lignanamides, two compound classes characteristic of this species. The chromatographic workflow enabled the separation of multiple peaks, whose identification was supported by UV–Vis absorption profiles and MS/MS fragmentation patterns (Supplementary file). Table 2 summarizes the retention times, experimental m/z values, molecular formulas, and MS² fragmentation patterns of the phenolic constituents detected in the ethyl acetate fraction along with their putative identifications and quantitative levels, providing a comprehensive overview of the phenolic composition of this fraction.

Table 2: Retention time (Rt), experimental mass (m/z), molecular formula, MS² fragment ions, identification and semi-quantification of the phenolic compounds in the ethyl acetate fraction of defatted cannabis seeds (*Cannabis sativa* L.)

Phenolic class	Rt (min)	[M – H] [–] (m/z)	Formula	MS ² fragments	Phenolic compound	Concentration (mg g ^{–1} extract)
Phenolic acids	13.9	121.0375	C ₇ H ₆ O ₂	Not fragmented	Benzoic acid	15.83 ± 0.57
	16.1	163.0481	C ₉ H ₈ O ₃	119 (100), 93 (8)	<i>p</i> -coumaric acid	0.35 ± 0.01
	17.7	193.0588	C ₁₀ H ₁₀ O ₄	134 (100), 178 (37), 149 (13)	Ferulic acid	0.58 ± 0.001
					Total	16.76 ± 0.57
Hydroxy-cinnamic acid amides	19.2	298.1161	C ₁₇ H ₁₇ NO ₄	135(100), 178(46), 298(45), 161(25), 136(12), 284(6)	<i>N</i> -trans-caffeoyltyramine isomer ^a	5.41 ± 0.13
	20.8	298.1159	C ₁₇ H ₁₇ NO ₄	135(100), 178(43), 161(42), 136(30), 298(16), 256(5), 148(5)	<i>N</i> -trans-caffeoyltyramine ^a	167.44 ± 2.50
	24.2	282.1218	C ₁₇ H ₁₇ NO ₃	145(100), 119(85), 282(54), 162(46), 134(14), 240(10)	<i>N</i> -trans-coumaroyltyramine ^a	6.90 ± 1.04
	24.9	312.1322	C ₁₈ H ₁₉ NO ₄	178(100), 135(45), 297(46), 312(45), 148(12)	<i>N</i> -feruloyltyramine ^a	54.24 ± 0.49
	29.1	582.2691	C ₃₄ H ₃₇ N ₃ O ₆	462(100), 582(87), 342(76), 316(10), 436(11), 299(4), 217(2), 533(2)	Tri- <i>p</i> -coumaroylspermidine ^a	6.91 ± 0.36
					Total	240.90 ± 4.51
	23.4	593.2014	C ₃₄ H ₃₀ N ₂ O ₈	593(100), 454(27), 639(21), 523(16), 536(13), 428(11), 482(11)	Cannabisin A ^a	81.21 ± 7.39
	23.7	595.2148	C ₃₄ H ₃₂ N ₂ O ₈	432(100), 595(93), 485(68), 269(33), 322(30)	Cannabisin B ^a	89.91 ± 2.38
	24.6	595.2151	C ₃₄ H ₃₂ N ₂ O ₈	416(100), 595(99), 269(46), 432(45), 458(30), 485(29), 295(23)	Cannabisin B Isomer 1 ^a	30.09 ± 1.27
	25.3	595.2164	C ₃₄ H ₃₂ N ₂ O ₈	485(100), 432(40), 322(33), 269(20), 348(9), 456(8), 595 (6)	Cannabisin B Isomer 2 ^a	4.16 ± 0.36

Lignanamides	25.9	609.2324	C ₃₅ H ₃₄ N ₂ O ₈	283(100), 446(66), 268(26), 609(9), 377(4)	Demethylgrossamide ^a	6.19 ± 0.16
	26.4	609.2321	C ₃₅ H ₃₄ O ₈ N ₂	499(100), 609(95), 446(70), 447(27), 336(20), 269(5)	Cannabisin C ^a	27.48 ± 0.32
	26.9	609.2332	C ₃₅ H ₃₄ O ₈ N ₂	446(100), 485(66), 609(21), 322(19), 472(7), 279(6), 499(6)	Cannabisin C isomer ^a	10.11 ± 0.57
	28.5	623.2479	C ₃₆ H ₃₆ N ₂ O ₈	460(100), 623(94), 283(35), 268(7), 444(9), 499(4)	Cannabisin D ^a	9.18 ± 0.28
	28.9	595.2168	C ₃₄ H ₃₂ N ₂ O ₈	432(100), 269(99), 458(36), 595(22), 295(10), 338(7), 250(2)	3,3-didemethylgrossamide ^a	29.17 ± 0.21
	29.8	641.2579	C ₃₆ H ₃₈ N ₂ O ₉	623(100), 489(90), 281(65), 431(40), 641(30), 591(15), 460(11), 312(12)	Cannabisin E ^a	7.17 ± 0.30
	30.2	490.1957	C ₂₈ H ₂₉ NO ₇	472(100), 490(43), 460(34), 488(2)	Grossamide K ^a	1.54 ± 0.03
	31.3	595.2147	C ₃₄ H ₃₂ N ₂ O ₈	298(100), 595(57), 430(17), 427(5), 547(4)	Cannabisin M ^a	28.87 ± 0.56
	31.5	595.2168	C ₃₄ H ₃₂ N ₂ O ₈	107(100), 298(49), 595(23)	3,3'-demethyl-heliotropamide ^a	9.27 ± 0.04
	31.7	892.3164	C ₅₁ H ₄₇ N ₃ O ₁₂	430(100), 595(85), 593(55), 727(24), 485(11), 322(11), 892(9)	Unnamed condensed trilignanamide ^b	14.39 ± 0.27
	32.0	595.2154	C ₃₄ H ₃₂ N ₂ O ₈	298(100), 595(42), 296(7), 178(1)	Cannabisin Q ^a	8.58 ± 0.10
	32.3	623.2478	C ₃₆ H ₃₆ N ₂ O ₈	460(100), 623(61), 297(35), 486(29), 352(5)	Cannabisin F ^a	8.60 ± 0.10
	33.4	609.2298	C ₃₅ H ₃₄ N ₂ O ₉	609(100), 296(41), 312(25), 417(17), 446(5), 176(4), 581(3)	Isocannabisin N ^c	5.71 ± 0.18
	34.2	623.2485	C ₃₆ H ₃₆ N ₂ O ₈	623(100), 460(77), 591(47), 297(32), 471(30), 551(23), 432(17), 486(15), 428(11), 282(11)	Grossamide ^a	46.86 ± 1.23

	34.5	934.3641	C ₅₄ H ₅₃ N ₃ O ₁₂	Not Fragmented	Cannabisin O ^d	4.08 ± 0.43
	35.4	589.2366	---	426 (100), 261(10), 443(8), 589(7), 255(7), 279(5), 163(5)	Unnamed lignanamide ^d	26.78 ± 0.18
					Total	449.34 ± 14.05

Except for phenolic acids, all phenolic concentrations were expressed in mg caffeoyltyramine equivalent per gram of extract. Results are the mean of three replicates and presented as mean ± SD. ^a [12], ^b [33], ^c [30], ^d [18]

Five hydroxycinnamic acid amides were identified, including two isomers of *N-trans*-caffeoyltyramine, *N*-feruloyltyramine, *N-trans*-coumaroyltyramine, and tri-*p*-coumaroylspermidine. These compounds exhibited UV maxima characteristic of conjugated cinnamic acid derivatives, while their MS/MS spectra showed characteristic fragment ions produced by amide-bond cleavage, typically yielding tyramine- or spermidine-derived fragments. *N-trans*-caffeoyltyramine was assigned to two detected constituents based on their nearly identical mass spectrometric profiles. Both exhibited deprotonated ions at m/z 298.1161 and 298.1159 and their MS² spectra featured the same dominant fragment ions at m/z 135, 161, and 178. This fragmentation pattern corresponds to the characteristic loss of the tyramine moiety (yielding the base peak at m/z 135) and the N-C α bond cleavage generating the ion at m/z 178 [12]. Furthermore, their UV absorption profiles closely matched those typically reported for *N-trans*-caffeoyltyramine [34], supporting their annotation as *N-trans*-caffeoyltyramine and its geometrical isomer. Similarly, the compound identified as *N*-feruloyltyramine showed a deprotonated molecular ion at m/z 312.1322 and generated fragment ions at m/z 178, 135, 297, and 148, a pattern consistent with previously reported data for this molecule [13,33]. The simultaneous presence of m/z 178 (feruloyl unit) and m/z 135 (tyramine unit) reflects the characteristic amide bond cleavage of this phenylamide.

The second and most abundant group consisted of lignanamides, a hallmark of *Cannabis* seed chemistry. In total, 20 compounds were identified including cannabisin A, B (three isomers), C (two isomers), D, E, F, M, Q, O, in addition to isocannabisin N, demethylgrossamide, 3,3-didemethylgrossamide, grossamide K, 3,3-demethyl-heliotropamide, grossamide, and two unnamed lignanamides. Their identification was supported by parent ions falling within the expected mass range and fragmentation behaviors characteristic of dilignol skeletons linked to phenylalkylamine units. Cannabisin A, for example, was identified by its deprotonated molecular ion at m/z 593.2014. Its MS² spectrum

displayed characteristic fragment ions at m/z 454, 523, 536, 428, and 482. Its UV absorption maximum at 255 nm is in agreement with previously reported data [34]. Similarly, cannabisin B was assigned to a metabolite exhibiting a deprotonated ion at m/z 595.2148 and fragment ions at m/z 432, 485, 269, and 322. These ions are likely due to cleavage of the amide-linked side chains and losses of aromatic moieties, with m/z 432 likely arising from the loss of HNCO together with a *p*-hydroxystyrene moiety, m/z 485 possibly corresponding to a loss of a catechol unit, and m/z 322 formed by the sequential loss of a ~163 Da together with a catechol unit [12]. Two additional compounds displaying nearly identical mass spectral and UV profiles were interpreted as geometrical isomers of cannabisin B.

The concentrations of the individual phenolic compounds identified in the ethyl acetate fraction are summarized in Table 2. Compounds are grouped by phenolic class: phenolic acids, hydroxycinnamic acid amides (HCAAs), and lignanamides. Lignanamide were the most abundant class overall, totaling 449.34 mg CTE g⁻¹ of extract, with Cannabisin B (89.91 mg CTE g⁻¹ extract) and Cannabisin A (81.21 mg CTE g⁻¹ extract) as the dominant constituents. HCAAs formed the second most concentrated class (240.90 mg CTE g⁻¹ extract), primarily dominated by *N-trans*-caffeoyltyramine (167.44 mg g⁻¹) and *N-feruloyl*tyramine (54.24 mg CTE g⁻¹ extract). Phenolic acids were present at lower levels (16.76 mg g⁻¹) with benzoic acid being the most major component in this group (15.83 mg g⁻¹). Overall, the chemical profile showed that the ethyl acetate fraction efficiently concentrates mid- to high-polarity phenolic constituents, particularly lignanamides and HCAAs. These molecules are well known for their antioxidant, anti-inflammatory, and enzyme-inhibitory properties, which likely contribute to the fraction's biological effects. The structural diversity of the identified molecules further highlights the metabolic uniqueness of *Cannabis sativa* seeds, underscoring this matrix as an underexplored yet promising source of bioactive phenolics.

3-3-2- Elastase and collagenase inhibition activities

Elastase and collagenase inhibition are important targets in the development of anti-aging agents, as these enzymes play central roles in the degradation of dermal collagen and elastin fibers. Despite this relevance, studies investigating the inhibitory effects of cannabis-derived extracts on these enzymes remain limited. In our study, the ethyl acetate fraction obtained from cannabis seeds exhibited an IC_{50} of $31.05 \mu\text{g mL}^{-1}$ against elastase, a value significantly ($p < 0.05$) lower than that of the positive control, quercetin ($39.23 \mu\text{g mL}^{-1}$). In contrast, for collagenase inhibition, the ethyl acetate fraction showed an IC_{50} of $75.53 \mu\text{g mL}^{-1}$, which was significantly higher ($p < 0.05$) than epigallocatechin gallate ($25.83 \mu\text{g mL}^{-1}$) (Table 3).

Table 3: IC_{50} results of enzyme inhibitory activity of ethyl acetate fraction from defatted cannabis seed extracts and corresponding positive controls.

	Ethyl acetate fraction	Positive control *
Elastase inhibition	31.05 ± 2.32 a	39.23 ± 2.43 b
Collagenase inhibition	75.53 ± 2.81 b	25.83 ± 1.81 a
Lipoxygenase inhibition	5.71 ± 0.30 b	1.19 ± 0.02 a
Tyrosinase inhibition	141.72 ± 7.91 b	14.04 ± 0.93 a

Different letters in each row mean statistically different (p -value < 0.05) based on student test. * Quercetin for elastase and lipoxygenase inhibition, epigallocatechin gallate for collagenase inhibition, and kojic acid for tyrosinase inhibition assay. All results were expressed in $\mu\text{g mL}^{-1}$

Our findings are particularly promising, as they suggest that the ethyl acetate fraction contains compounds capable of inhibiting elastase and collagenase. These bioactive constituents are predominantly phenolic compounds, as evidenced by the quantitative data obtained from HPLC-DAD/ESI-MS² analysis (Table 2). However, further in-depth analyses are needed to refine the fractionation and isolate individual molecules, allowing for a more precise assessment and confirmation of their specific biological activities.

To our knowledge, the only study specifically investigating elastase and collagenase inhibitory activities of hemp seeds is that of Michailidis et al. [35], although their evaluation

was conducted at three fixed concentrations rather than IC_{50} determination. These authors reported weak anti-elastase activity relative to our results, with inhibition remaining below 40% even at a high extract concentration ($300 \mu\text{g mL}^{-1}$), compared with the positive control elastinal ($IC_{50} = 0.5 \mu\text{g mL}^{-1}$). In contrast, they observed good anti-collagenase activity for all tested extracts, reaching up to 98.5% inhibition at $600 \mu\text{g mL}^{-1}$ (positive control phosphoramidon: $IC_{50} = 16 \mu\text{M}$) [35].

Other parts of the cannabis plant have demonstrated even weaker elastase inhibition. Zagórska-Dziok et al. [36] recorded only 30% inhibition at 1 mg mL^{-1} for leaf extracts, while Mastellone et al. [37] observed no elastase inhibition at $166 \mu\text{g mL}^{-1}$ using aerial part extracts. In contrast, our results revealed markedly higher elastase inhibition, highlighting the potential of seed-derived phenolics as promising bioactive molecules. Collectively, the current evidence underscores a research gap in the exploration of cannabis seed bioactive compounds targeting elastase and collagenase, and emphasizes the need for more detailed biochemical studies. Moreover, the link between the structural features of phenylpropanoid amides and their inhibitory effects on elastase or collagenase remains largely unexplored. This gap in structural understanding underscores the need for targeted investigations to identify the molecular determinants underlying their anti-elastase and anti-collagenase activities.

3-3-3- Lipoxygenase inhibition activity

Lipoxygenases (LOX) play a central role in the formation of pro-inflammatory lipid mediators, making their inhibition an important strategy for mitigating oxidative stress and inflammation in the skin and other tissues. Numerous studies have examined the LOX-inhibitory properties of *Cannabis sativa* tissues, including leaves, stems, sprouts, inflorescences, and isolated cannabinoids such as cannabidiol and Δ^9 -tetrahydrocannabinol [38–41]. In contrast, investigations specifically targeting LOX inhibition by cannabis seeds remains limited, despite their unique phytochemical profile. This shortage of data highlights

the importance of evaluating seed-derived fractions as potential alternative sources of anti-inflammatory agents.

Many LOX bioassays, including the present study, investigate inflammatory processes using plant-derived lipoxygenases, notably soybean lipoxygenase [22,42,43]. These enzymes are well-established and validated models for investigating lipid peroxidation pathways relevant to human physiology. Humans express multiple LOX isoforms, that generate pro-inflammatory lipid mediators, and excessive activation of these pathways contributes to oxidative stress and cutaneous inflammation. Therefore, assessing the ability of cannabis seed extracts to inhibit soybean LOX offers an indirect yet informative indication of their potential to modulate inflammation-related lipid oxidation processes in human skin.

As shown in Table 3, the ethyl acetate fraction of the cannabis seed extract exhibited substantial lipoxygenase inhibitory activity, with an IC_{50} of 5.71 $\mu\text{g/mL}$. Quercetin, included as the positive control, demonstrated stronger inhibition ($IC_{50} = 1.19 \mu\text{g/mL}$). The anti-inflammatory potency of our EA fraction exceeds that reported for many crude plant extracts [42–44], whose higher IC_{50} values often reflect lower levels of active anti-inflammatory constituents. This result aligns with the enrichment of phenolic compounds, particularly HCAA and lignanamides, achieved through solvent partitioning (Table 2) and shows that this bioactive seed-derived fraction can effectively modulates lipid peroxidation pathways linked to inflammation.

Interestingly, Ali and Akhtar [45] provided complementary clinical evidence supporting the anti-inflammatory potential of cannabis seed extracts, although do not directly demonstrate LOX inhibition. In their clinical study, a 3 % cannabis seed extract cream applied twice daily for 12 weeks significantly reduced cheek sebum levels and erythema without causing irritation. These outcomes suggest that compounds derived from cannabis seeds can influence cutaneous lipid regulation and inflammatory processes. Such effects are

mechanistically compatible with partial modulation of the LOX pathway, as LOX inhibition decreases the formation of pro-inflammatory leukotrienes, such as LTB₄, which contribute to erythema and enhance sebocyte activity. In a recent *in vitro* study, Kongtananeti et al. [40] reported an IC₅₀ value of 9.44 µg/mL for 5-LOX inhibitory activity of an ethyl acetate cannabis seed extract, reflecting slightly weaker potency than observed in our result. It is important to note that both of the referenced studies employed a whole seed extract, which likely contained the lipid-rich fraction (oil) and its associated fatty acids, potentially influencing their bioactivity profiles.

Indeed, several studies have already demonstrated the anti-inflammatory activity of cannabis seed oil. For example, Tammaro et al. [46] showed that a topical formulation containing cannabis seed oil helped relieve inflammatory skin conditions such as psoriasis and dermatitis. Their findings suggest that products enriched with hemp seed oil could serve as safe and effective complementary options alongside steroid-based treatments. Similarly, Jin and lee [47] reported that a hexane extract of hemp seeds (representing the oil fraction) inhibited 5-lipoxygenase activity in sebocytes and reduced *Propionibacterium acnes*-induced inflammation by suppressing Nuclear factor-kappa B/ Mitogen-Activated Protein Kinase (NF-κB/MAPK) signaling pathways and key pro-inflammatory cytokines. Together, these studies underscore the value of hemp seed- derived ingredients in managing skin inflammation, while our results highlight the more specialized dermatological potential of phenolic-rich fractions obtained from defatted cannabis seeds, an area that has not been previously examined.

However, the structural determinants governing lipoxygenase inhibition by cannabis-derived phenolics (particularly phenylpropanoid amides) are still not fully understood. The influence of specific chemical attributes, such as the extent of prenylation, patterns of aromatic substitution, and variations in amide side-chain structure on inhibitory potency remains unclear. This knowledge gap highlights the need for more targeted Structure-Activity

Relationship (SAR) studies to clarify the molecular features that govern lipoxygenase inhibition within this class of natural compounds.

3-3-4- Tyrosinase inhibition activity

Tyrosinase inhibitors have attracted growing interest in recent years because of their ability to prevent skin hyperpigmentation disorders and to reduce enzymatic browning in plant-based products, such as fruits and vegetables [48]. Consequently, they are widely used across the cosmetic, pharmaceutical, and food industries. The demand for skin-lightening products is especially notable, with an estimated 15% of the global population using or seeking such treatments [49].

In our study, the ethyl acetate fraction displayed notable tyrosinase inhibitory activity with an IC_{50} value of $141.72 \mu\text{g mL}^{-1}$, indicating the presence of constituents with anti-tyrosinase effects. For comparison, the reference inhibitor kojic acid exhibited a much lower IC_{50} value ($14.04 \mu\text{g mL}^{-1}$) (Table 3). A previous investigation on extracts from cannabis aerial parts reported tyrosinase inhibition rates of only 33%-38% at $331 \mu\text{g mL}^{-1}$ [37], a markedly weaker response than that observed in our seed extracts. This contrast underscores the superior bioactivity of cannabis seeds and their promise as a natural source of tyrosinase inhibitors. However, research on the anti-tyrosinase activity of cannabis seed extracts or their isolated phenolic compounds remains scarce, with most existing studies centered on “synthetic” derivatives of known phenolic amides.

The first report on the tyrosinase-inhibitory effects of cannabis seed extracts appeared in 2019, where the authors observed activity with an IC_{50} of 0.07 mg mL^{-1} , approximately 14-fold weaker than kojic acid (IC_{50} of 0.005 mg mL^{-1}) [28]. In contrast, Michailidis et al. [35] evaluated multiple extraction techniques and different concentrations of hemp seed extracts but observed no more than 20% tyrosinase inhibition, even at $500 \mu\text{g mL}^{-1}$, while kojic acid

displayed an IC_{50} of just 50 μM (7.11 $\mu g mL^{-1}$). Another study using an ethyl acetate fraction of cannabis seeds reported an IC_{50} of 60.39 $\mu g mL^{-1}$, approximately 13 times higher than that of kojic acid (4.7 $\mu g mL^{-1}$) [50]. In our findings, the IC_{50} value of the ethyl acetate fraction was only ten times higher than kojic acid, indicating stronger-performance relative to previous findings. In addition, a recent investigation reported extremely weak anti-tyrosinase activity with an IC_{50} value greater than 1000 mg/mL for a crude ethyl acetate extract from cannabis seeds [40], underscoring the crucial role of fractionation in enhancing bioactivity.

Interestingly, several phenolic amides isolated from cannabis seeds have demonstrated promising anti-tyrosinase potential. Rea et al. [50] reported that *N-trans*-caffeoyltyramine and *N-trans*-coumaroyltyramine exhibited IC_{50} values of 8.13 and 11.78 $\mu g mL^{-1}$, respectively. In another study among nine phenylpropanoid amides isolated from hemp seed hulls, cannabisin A and cannabisin B demonstrated strong tyrosinase inhibition (3.3 and 8.1 μM respectively), both surpassing the reference compound kojic acid (13.5 μM). Moreover, cannabisin A not only inhibited tyrosinase *in vitro*, but also significantly reduced melanin content and tyrosinase activity in B16F10 melanoma cells, underscoring its promise as a cosmetic ingredient [51]. Kinetic analyses and docking simulations, further revealed that Cannabisin A functions as a non-competitive inhibitor by binding near the active site, whereas Cannabisin B acts competitively through direct interaction with the enzyme's catalytic site [51].

In parallel, several studies have explored synthetic hydroxycinnamic acid amides (HCAAs) as potential tyrosinase inhibitors. For example, some *N*-(phenylalkyl)cinnamides synthesized by coupling of cinnamic acid or its derivatives with phenylalkylamines were evaluated for anti-tyrosinase activity, and two compounds exhibited inhibitory effects comparable to the reference compound hydroquinone [52]. Additionally, phenylpropanoid amides synthesized from octopamine were shown to be more potent tyrosinase inhibitors than their dopamine-derived counterparts, with one derivative achieving an IC_{50} of 5.3 μM [53].

Interestingly, octopamine derivatives have previously been identified in certain cannabis seeds, unlike dopamine-derived analogues [12]. In the same context, amongst various synthesized phenylpropanoid amides, *p*-coumaroyldopamine, *p*-coumaroyltyramine and their corresponding amino acid precursors demonstrated the strongest tyrosinase-inhibitory activities [48]. Moreover, three synthesized HCAA (*N-trans*-caffeoyltyramine, *N-trans*-coumaroyltyramine, and *N-trans*-feruloyltyramine) were assessed using both *in vitro* and *in vivo* anti-tyrosinase inhibition models, with *N-trans*-caffeoyltyramine showing the greatest efficacy [24]. Notably, *N-trans*-caffeoyltyramine achieved complete tyrosinase inhibition at 0.1 mM ($28\mu\text{g mL}^{-1}$) in an earlier study [54]. The same authors also discuss how the chemical structure of phenolic amides shapes their inhibitory potential. Key structural determinants include amide bond formation, the spacing between functional groups, and the presence and position of hydroxyl groups. Notably, coupling tyramine to caffeic acid or related compounds through an amide bond results in markedly stronger activity, whereas the individual components alone (e.g., tyramine, dopamine, caffeic acid, ferulic acid) exhibit only weak effects highlighting the importance of the amide linkage. Optimal spacing, with two carbon atoms separating the amide group from the aromatic rings, also appears crucial for better interaction with the enzyme. Additionally, the presence of a catechol group (two hydroxyls on the aromatic ring), particularly on the acid moiety, further enhances the activity [54]. Consistent with these observations, Le Mellay-Hamon and Criton [52] emphasized that hydroxylation at the R1 position on the hydroxycinnamic moiety significantly modulates activity. In the same context, Yu and Fan [55] reported a synergistic effect when combining 4-hydroxycinnamic and ferulic acid, further underscoring how spatial and chemical features jointly contribute to optimal enzyme inhibition.

Taken together, these results suggest that the bioactivity of our ethyl acetate extract is largely driven by its enriched content of hydroxycinnamic acid amides and lignanamides,

which were concentrated through fractionation, and particularly to the presence of cannabisin A, cannabisin B, and *N-trans*-caffeoyltyramine.

Conclusion

Beyond their demonstrated *in-vitro* antioxidant potential, hemp seed extracts also exhibit anti-collagenase, anti-elastase, anti-tyrosinase, and anti-inflammatory activities, offering a multifunctional approach to skin care. By mitigating oxidative stress, preventing the degradation of elastin and collagen fibers, inhibiting melanin synthesis, and modulating the inflammatory processes, these extracts show strong promise as a natural skin-brightening, anti-aging, and anti-inflammatory agents for cosmetic formulations. These findings emphasize the need to reconsider cannabis seeds as a valuable source of tyrosinase, elastase, collagenase, and lipoxygenase inhibitors and underscore the importance of further exploring their cosmetic applications. To further substantiate these results, future research should explore the molecular mechanisms underlying enzyme inhibition. In particular, molecular docking and dynamic simulations could provide deeper insight into the interactions between the identified phenolic compounds and skin-related enzymes. In parallel, the isolation of individual phenolic constituents and the elucidation of their structure-activity relationships would allow a clearer understanding of the compounds responsible for the observed bioactivities. Finally, *in vivo* investigations and well-designed clinical trials are essential to validate the biological relevance of these findings and to support their translation into practical cosmetic applications.

Conflicts of interest

No conflicts of interest are declared.

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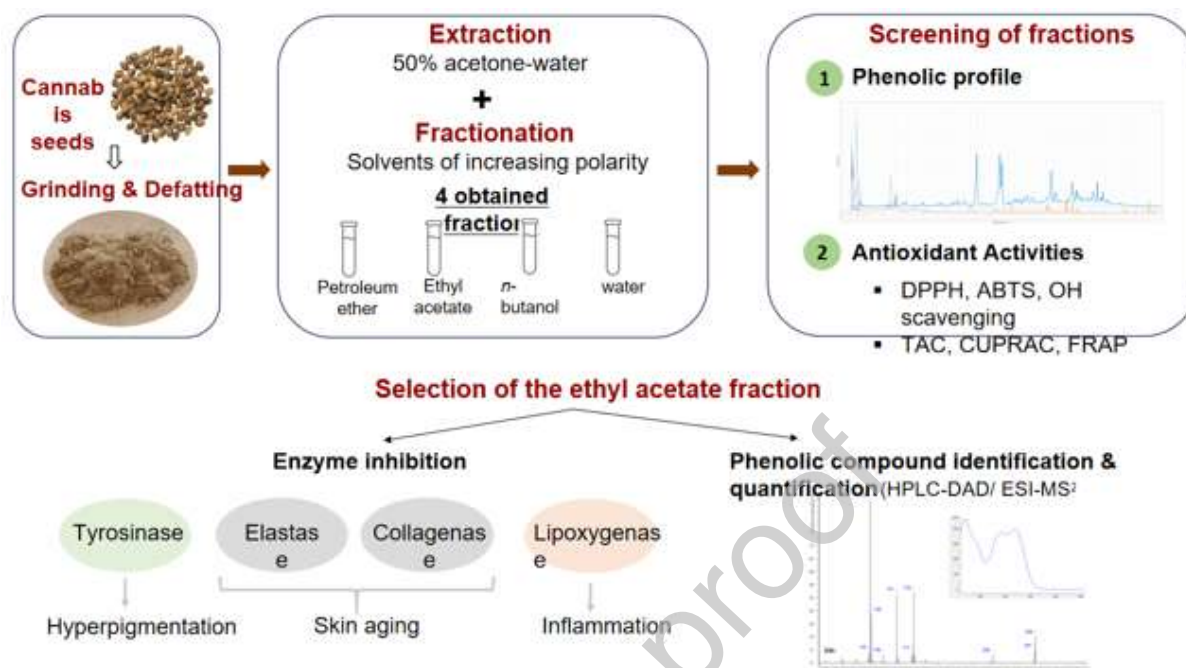
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Graphical abstract



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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: