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Research Article

Boosting Biological Properties of Red Chili Pepper Phenolics Using Different Varieties Mixture of Algerian Local Cultivars

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ABSTRACT

This study aimed to identify bioactive compounds contained in methanolic extract (MERCPM) and aqueous extracts prepared by infusion (AEIRCPM) and decoction (AEDRCPM) of four *Capsicum annuum* varieties: *C. annuum var*. Jalapeno, *C. annuum var*. Baklouti, *C. annuum var*. Black Prince and *C. annuum var*. Black Cobra and to evaluate their antioxidant and anti-inflammatory activities. Liquid chromatography coupled to mass spectrometry (LC-MS) analysis was employed to quantify and identify phenolic compounds in red chili pepper extracts. Antioxidant activity was assessed using the DPPH free radical scavenging method and ferric reducing antioxidant power, while the anti-inflammatory effect was evaluated using the inhibition of albumin bovine denaturation method. Three major compounds were identified in MERCPM: catechin, capsaicin, and dihydrocapsaicin. While capsaicin, syringic acid, and dihydrocapsaicin were the major phenolics in AEIRCPM, and vanillic acid followed by capsaicin, syringic acid, trans-ferulic acid, and dihydrocapsaicin in AEDRCPM, which highlighted the potential of red chili pepper extracts as source of bioactive substances. All extracts demonstrated significant antioxidant effect, with DPPH free radicals' inhibition efficiency exceeding 50% using AEDRCPM, achieving an IC_{50} of 2.09 mg/mL and IC_{100} of 3.84 mg/mL. Anti-inflammatory testing revealed strong and significant effects noted for all chili extracts exceeding 80% at 15 mg/mL. The findings underscore the potential of the fourth varieties mixture of red chili pepper as natural remedies warranting further investigation into its pharmacological and therapeutic applications in preventing diseases related to oxidative stress and inflammation.

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1. INTRODUCTION

The scientific community has increasingly encouraged the public to pay closer attention to daily dietary habits, as diet plays an important role in maintaining health and preventing diseases, particularly those associated with oxidative stress and inflammation. In this context, functional foods and natural food supplements have gained considerable interest because they contain bioactive compounds that may contribute to disease prevention and overall well-being. Among these, red chili pepper, scientifically known as *Capsicum annuum*, is regarded as an important dietary component with both nutritional and medicinal value. This species originated in South America, where it has long been used for culinary purposes as well as in traditional medicine (Singletary, 2011). The term “chili pepper” generally refers to 27 species of pungent fruits belonging to the genus *Capsicum* within the family Solanaceae. Of these, five species are widely cultivated, namely *Capsicum annuum*, *C. baccatum*, *C. pubescens*, *C. chinense*, and *C. frutescens*. Among them, *C. annuum* is the most extensively distributed and exhibits the greatest genetic diversity, making it one of the most significant and economically important species in the genus (Haq et al., 2022).

Chili peppers currently serve several beneficial functions for human health. They contain various bioactive compounds such as vitamin C, carotenoids, tocopherols, flavonoids, phenolics, and capsaicinoids, which are used for therapeutic purposes, as food flavorings, or coloring agents (Kantar et al., 2016; Mohd Hassan et al., 2019; Razola-Díaz et al., 2022). Phenolic compounds in chili pepper significantly enhance the nutritional and functional value of these spices (Lahbib et al., 2023). The phenolic profile of peppers is primarily composed of flavonoids (quercetin, luteolin, and apigenin), along with chlorogenic acid, caffeic acid, and ferulic acid, which contribute to immune modulation and cardiovascular protection (Jeong et al., 2011). These compounds also exhibit strong antioxidant properties, effectively scavenging reactive oxygen species (ROS) that are implicated in the development of chronic diseases (González Paramás et al., 2019). Red chili pepper has been also used for the treatment of cough, toothache, sore throat, parasitic infections, rheumatism, wound healing, and also utilized as an antiseptic, counterirritant, appetite stimulator (Singletary, 2011).Thus, capsaicinoids are the unique phytochemical group found exclusively in peppers, classified as both phenolics and alkaloids (Mazourek et al., 2009). These compounds are responsible for the burning sensation in chili pepper and are exploited in culinary applications, personal safety, and pain management (Simon & De-Araujo, 2005; Willis, 2009; Haar et al., 2017; Fernández-Carvajal et al., 2022).

Topically, capsaicin is widely used for its analgesic properties in managing chronic pain conditions, such as lower back pain, by desensitizing sensory neurons and reducing the transmission of pain signals (Arora et al., 2021). It's utilized for the treatment of psoriasis and rheumatoid arthritis, diabetic neuropathy, postherpetic neuralgia (PHN), cluster headache, post-mastectomy pain syndrome (PMPS), reflex sympathetic dystrophy, dermatitis and eczema itching, postoperative nausea and vomiting, bladder hyperactivity, gallstone, anorexia, hemorrhoids and liver congestion. It's also effective against foodborne gastrointestinal pathogens including *Listeria monocytogenes*, *Salmonella typhimurium* and *Bacillus cereus*, tonsillitis and rhinitis and fibromyalgia. Besides, capsaicin is used as pesticide in agri-food industries, as analgesic, for its anti-obesity, antihypertensive, anti-arrhythmic and anti-ischemic properties, and as gastroprotective agent. It can stimulate saliva and digestive pancreas enzymes, small intestine, and also stimulate hair growth in alopecia areata (spot baldness). Moreover, anticoagulant activity, prevention of aspiration pneumonia, protecting neuromuscular junctions from *Clostridium botulinum* neurotoxin A and improving cognitive function are also attributed to capsaicin beneficial properties (Srinivasan, 2005; Hayman & Kam, 2008; Barceloux, 2009; Papoiu & Yosipovitch, 2010; Singletary, 2011; Pawar et al., 2011).

Red chili pepper is also recognized for its numerous biological properties, including potent antioxidant and anti-inflammatory effects, as well as anticancer, antimicrobial activities against various pathogenic strains, and an antiviral effect. Recently, capsaicinoids, particularly capsaicin, have been shown to manage the symptoms of significant viral diseases, including Covid-19 (Faisal & Mustapha, 2025). It's commonly used in the treatment of conditions such as gastritis, rheumatism, as well as some intestinal problems (Rosa et al., 2002; Janssens et al., 2013; Friedman et al., 2018). Red pepper as a drug is given in atonic dyspepsia and flatulence due to motility increasing in the gastric antrum, duodenum, proximal jejunum and colon (Maji & Banerji, 2016). Moreover, Chilies are known to protect against gastrointestinal ailments including dyspepsia, loss of appetite, gastro-esophageal reflux disease and gastric ulcer due to the several mechanisms such as reducing the food transition time through the gastrointestinal tract and anti-*Helicobacter pylori* effects (Low, 2006; Kim et al., 2014). Thus, the primary objective of this study was to valorize the bioactive compounds extracted from a mixture of different red chili pepper varieties (*C. annuum*) collected from northwestern Algeria, and to systematically evaluate their antioxidant and anti-inflammatory activities.

2. METHODOLOGY

2.1. Plant Material

Four varieties of *C. annuum* were used in this study. The fruits were collected from the Belarbi region, Sidi Bel Abbès, in north-western Algeria, in September 2023. The selected varieties included *C. annuum* var. Jalapeño, var. Baklouti, var. Black Prince, and var. Black Cobra. Botanical identification was based on the classification provided by the cultivating farmer. The fresh fruits were air-dried at ambient temperature and subsequently ground into a fine powder, which was used for the preparation of phenolic extracts.

2.2. Phenolic Extracts Preparation

Phenolic compounds were extracted following a modified methanolic extraction protocol (Romani et al., 2006). Briefly, 2.5 g of each red chili pepper variety was weighed and combined to obtain a total of 10 g of powdered sample. The mixture was extracted with 100 mL of 80% methanol (v/v) and subjected to maceration at room temperature for 24 h under continuous agitation. The resulting mixture was filtered using Whatman filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The obtained methanolic extract (MERCPM) was stored in sealed glass vials at 4°C to minimize oxidation. Aqueous extracts were prepared by infusion and decoction according to a previously described method (Chavane et al., 2001). For decoction, 10 g of the powdered sample was boiled with 100 mL of distilled water at 100°C for 30 min. For infusion, 100 mL of boiling distilled water was added to 10 g of sample and allowed to stand for 30 min. The mixtures were subsequently filtered, and the filtrates were dried at 45°C to obtain powdered aqueous extracts, namely AEIRCPM (infusion) and AEDRCPM (decoction). The extraction yield (%) was calculated using the equation: Yield (%) = (m_2 / m_1) × 100, where m_1 represents the initial weight of plant material (g) and m_2 corresponds to the weight of the dried extract (g).

2.3. LC-MS Analysis

The extracts of *C. annuum* fruits (50 mg) were dissolved in 1.0 mL of methanol in Eppendorf tubes, followed by the addition of 1.0 mL of hexane. The mixture was sonicated in an ultrasonic bath and subsequently centrifuged at 9000 rpm for 10 min. An aliquot (100 µL) of the methanolic phase was collected and diluted with 450 µL of water and 450 µL of methanol. The final solution was filtered through a 0.45 µm membrane filter and transferred into LC vials for analysis. Quantitative analysis of natural compounds in both methanolic and aqueous extracts was performed using LC-ESI-MS/MS (Agilent Technologies 1260 Infinity II coupled with a 6460 Triple Quadrupole mass spectrometer). Separation was achieved on a reverse-phase column (Poroshell 120 EC-C18, 4.6 × 150 mm, 2.7 µm). Electrospray ionization (ESI) was operated in both positive and negative modes to detect mass-to-charge (*m/z*) ratios of the analytes. The column temperature was maintained at 40°C, and the injection volume was set at 4.0 µL. The mobile phase consisted of (A) water containing 0.1% formic acid and 5.0 mM ammonium formate, and (B) methanol containing 0.1% formic acid and 5.0 mM ammonium formate. The gradient elution program was as follows: 75% A (0-3 min), 50% A (4-12 min), 10% A (13-21 min), and 97.5% A (22-24 min). The flow rate was maintained at 0.4 mL/min. Nitrogen (N_2) was used as the nebulizing gas at a flow rate of 11 L/min, with a capillary voltage of 4000 V and a gas temperature of 300°C. Quantitative analysis was conducted using multiple reaction monitoring (MRM), where precursor and product ions, fragmentor voltages, and collision energies were optimized for each compound.

2.4. Phenolics Quantitative Determination

The total polyphenol content (TPC) was determined using the Folin-Ciocalteu method with slight modifications (Salleh & Ahmad, 2016; Gulcin & Alwasel, 2023). The results were expressed as milligrams of gallic acid equivalents per gram of dry RCPPE (mg GAE/g RCPPE), based on the gallic acid calibration curve ($Y = 0.122x + 0.055$; $R^2 = 1.000$). The total flavonoid content (TFC) was measured using the aluminium chloride ($AlCl_3$) colorimetric method (Shraim et al., 2021). The results were expressed as milligrams of quercetin equivalents per gram of dry RCPPE (mg QE/g RCPPE), using the calibration curve ($y = 0.049x - 0.023$; $R^2 = 0.974$). The total tannin content (TTC) was determined based on the vanillin assay, which involves the formation of a red chromophore complex through the reaction of vanillin with the aldehyde group at the C-6 position of the catechin A-ring, measured at 500 nm (Schofield et al., 2001). The results were expressed as milligrams of catechin equivalents per gram of dry RCPPE (mg CE/g RCPPE), according to the calibration curve ($Y = 0.177x - 0.192$; $R^2 = 0.989$).

2.5. Antioxidant Activity

2.5.1. DPPH Free Radical Scavenging

The DPPH radical scavenging assay was performed based on a previously reported method with slight modifications (Salleh & Ahmad, 2015). Briefly, 250 µL of red chili pepper extract at various concentrations (15-80 mg/mL) was mixed with 1 mL of freshly prepared ethanolic DPPH solution (0.1 mg/mL). A negative control containing only the DPPH solution was also prepared. The reaction mixtures were incubated in the dark at 20°C for 30 min. Absorbance was then measured at 517 nm using a spectrophotometer (Shimadzu UV mini-12400). The radical scavenging activity was calculated using the equation: Inhibition (%) = $((A_1 - A_2)/A_1) \times 100$, where A_1 represents the absorbance of the DPPH solution and A_2 represents the absorbance after addition of the extract or standard. The results were expressed as percentage inhibition of DPPH radicals, as well as IC_{50} and IC_{100} values derived from calibration curves (Yeo & Shahidi, 2019).

2.5.2. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power assay was performed according to a previously described method with slight modifications (Salleh & Ahmad, 2015). Briefly, 200 µL of red chili pepper extract at different concentrations (15-80 mg/mL) was mixed with 500 µL of phosphate buffer (0.2 M, pH 6.6) and 500 µL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated in a water bath at 50°C for 20 min. Subsequently, 500 µL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, 500 µL of the supernatant was mixed with 500 µL of distilled water and 100 µL of freshly prepared 0.1% ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm using a UV–Vis spectrophotometer (Shimadzu UV mini-1240) against a blank. Methanol was used as the negative control, while ascorbic acid served as the positive control. All measurements were carried out in triplicate (n = 3 ± SD), and an increase in absorbance indicated a higher ferric reducing power of the extracts.

2.6. Anti-inflammatory Activity

The protein denaturation inhibition assay was performed based on a previously reported method with slight modifications (Nithya & Parimala, 2026). Briefly, 1 mL of red chili pepper extract or standard (diclofenac) at different concentrations (15-80 mg/mL) was mixed with 1 mL of 1% bovine serum albumin (BSA) solution. The mixtures were first incubated at 37°C for 20 min, followed by heating in a water bath at 60°C for 3 min. After cooling, the absorbance was measured at 660 nm. All experiments were conducted in triplicate (n = 3 ± SD). The percentage inhibition of protein (bovine albumin) denaturation was calculated using the equation: Bovine albumin denaturation inhibition (%) = $[(Ac - As)/Ac] \times 100$; where Ac is the absorbance of the control (1% BSA solution) and As is the absorbance of the sample.

2.7. Statistical Analysis

All experiments were conducted in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics version 25. One-way and multivariate analysis of variance (ANOVA) were applied to evaluate differences among groups, with statistical significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Quantitative Determination of Total Polyphenols, Flavonoids and Tannins

The extraction yields and total polyphenol contents of the different methanolic and aqueous extracts of the red chili pepper varieties mixture are presented in Table 1. Maceration of the powdered sample in 80% hydromethanol resulted in the highest yield (32.48 ± 0.053%) compared to the aqueous extracts (Table 1). The choice of solvent plays a critical role in extraction efficiency and determines the classes of compounds recovered. Polar solvents, such as methanol, are particularly effective for extracting phenolic compounds due to their polarity. In addition, extraction parameters, including temperature and duration, must be optimized to maximize the recovery of bioactive constituents while minimizing potential degradation or interference with biological activity (Nisca et al., 2025). The higher yield observed for the hydromethanolic extract can be attributed to its suitable polarity, which enhances penetration into plant cell walls, facilitates cellular disruption, and promotes efficient solubilization of phenolic compounds. In contrast, infusion and decoction are conventional methods primarily used for extracting water-soluble constituents. The use of hot water and prolonged boiling (30 min) promotes the breakdown of plant tissues, thereby enhancing the release of polar compounds such as polyphenols, flavonoids, tannins, and saponins.

Table 1. Extraction yield (%) and quantitative determination of total polyphenols, flavonoids, and tannins in red chili pepper extracts (µg/g RCPPE)

Sample	**Aqueous extracts**		**Methanolic extract**
	AEIRCPM	**AEDRCPM**	**MERCPM**
Extraction Yield (%)	11.4±0.208	13.1±0.068	32.48±0.053
TPC (µg GAE/g RCPPE)	3033.94±22.28	2895.15±0.86	2601.21±0
TFC (µg QE/g RCPPE)	2146.26±4.81	2268.7±4.81	1982.99±14.43
TTC (µg CE/g RCPPE)	88.14±0.53	75.71±0	73.45±0

Values are expressed as mean ± standard deviation (n = 3). Differences were considered statistically significant at $p < 0.05$

All extracts exhibited high polyphenol contents, with slightly higher values observed in AEIRCPM and AEDRCPM, reaching 3033.94 ± 22.28 µg GAE/g and 2895.15 ± 0.86 µg GAE/g, respectively. All extracts were also rich in flavonoids, with concentrations exceeding 2000 µg QE/g, except for the methanolic extract, where the total flavonoid content (TFC) was 1982.99 ± 0 µg QE/g (Table 1). In addition, the highest tannin content was recorded in AEIRCPM (88.14 ± 0.53 µg CE/g). Mechraoui et al. (2021) suggested that variations in extraction yield are influenced by several factors, including extraction method, solvent polarity (with polar solvents generally providing higher yields), pH, temperature, extraction time, and the physicochemical properties of the sample. The presence of polyphenols, flavonoids, and catechin-type tannins was clearly demonstrated. These bioactive compounds are well known for their diverse biological activities, particularly antioxidant and anti-inflammatory effects (Salleh et al., 2019; Manso et al., 2021). Ghasemnezhad et al. (2021) also reported that different red chili pepper varieties are rich in polyphenols, especially flavonoids and tannins. These compounds play a crucial role in protecting human health by mitigating oxidative stress through the scavenging of free radicals, thereby reducing the risk of inflammatory diseases, cancer, and metabolic disorders (Chouhan & Singh, 2011).

Previous studies have reported comparable findings. For instance, Materska and Perucka (2005) documented total flavonoid contents in red peppers ranging from 1 to 15 mg QE/g extract. Lima et al. (2021) and Salamatullah et al. (2022) demonstrated that decoction is an effective method for extracting condensed tannins due to its ability to disrupt macromolecular complexes and release bound tannins. Ghasemnezhad et al. (2021) reported flavonoid contents ranging from 534.6 to 1423.8 mg QE/100 g dry extract, while total polyphenols ranged from 1367.5 to 3256.7 mg GAE/100 g dry weight. Tannin levels were comparatively lower, ranging from 8.4 to 58.9 mg CE/100 g dry weight. In comparison, the present study indicates relatively higher levels, highlighting the richness of the red chili pepper varieties mixture collected from the Belarbi region in north-western Algeria, with values exceeding 1000 µg/g RCPPE. Hassan et al. (2019) reported total phenolic contents of 10–17 mg GAE/g dry extract in aqueous extracts of *C. annuum*. Similarly, Faliarizao et al. (2025) reported polyphenol contents ranging from approximately 498.40 to 528.07 mg GAE/g dry extract using different extraction methods. Carvalho et al. (2015) reported a total phenolic content of 4.8 mg/g dry extract in selected *Capsicum* genotypes. Hudáková et al. (2023) observed total polyphenol contents ranging from 3.53 to 25.9 mg GAE/g dry extract, along with carotenoid contents between 114.7 and 1390.8 µg/g. Ornelas-Paz et al. (2009) demonstrated that heat treatments, particularly decoction, significantly increase flavonoid content in *C. annuum*.

Although tannins were present in lower amounts compared to flavonoids, their contribution remains significant due to their role in astringency and their involvement in antioxidant and antimicrobial activities (Araújo et al., 2008). Cruz-Hernández and Cuervo-Osorio (2025) further confirmed the richness of red chili pepper varieties in both capsaicinoids and polyphenols, correlating these compounds with strong antioxidant activity. Similarly, Jang et al. (2024) highlighted the abundant presence of polyphenols, flavonoids, and capsaicinoids contributing to the overall bioactivity of chili peppers. These findings suggest that the local *C. annuum* cultivars from Sidi Bel Abbès

(Belarbi region, Algeria) are rich sources of bioactive compounds with significant antioxidant potential and promising health benefits.

3.2. LC-MS Analysis

Results of the LC-MS analysis are presented in Tables 2-4 and Figure 1. In the hydromethanolic extract of the *C. annuum* varieties mixture, the major identified and quantified compounds were catechin (779.0452 µg/mL), followed by capsaicin (701.6327 µg/mL) and dihydrocapsaicin (563.5075 µg/mL). Other notable constituents included quercetin (226.4905 µg/mL), syringic acid (184.5894 µg/mL), quercetin-3-glucoside (159.3812 µg/mL), and salicylic acid (125.3260 µg/mL). In the aqueous extract prepared by infusion, capsaicin, syringic acid, and dihydrocapsaicin were the predominant compounds, with concentrations of 374.3950 µg/mL, 229.3785 µg/mL, and 193.5380 µg/mL, respectively. In contrast, the decoction extract showed vanillic acid as the major compound, with the highest concentration (930.6238 µg/mL), followed by capsaicin (328.1265 µg/mL), syringic acid (187.0210 µg/mL), trans-ferulic acid (129.7444 µg/mL), and dihydrocapsaicin (124.4608 µg/mL).

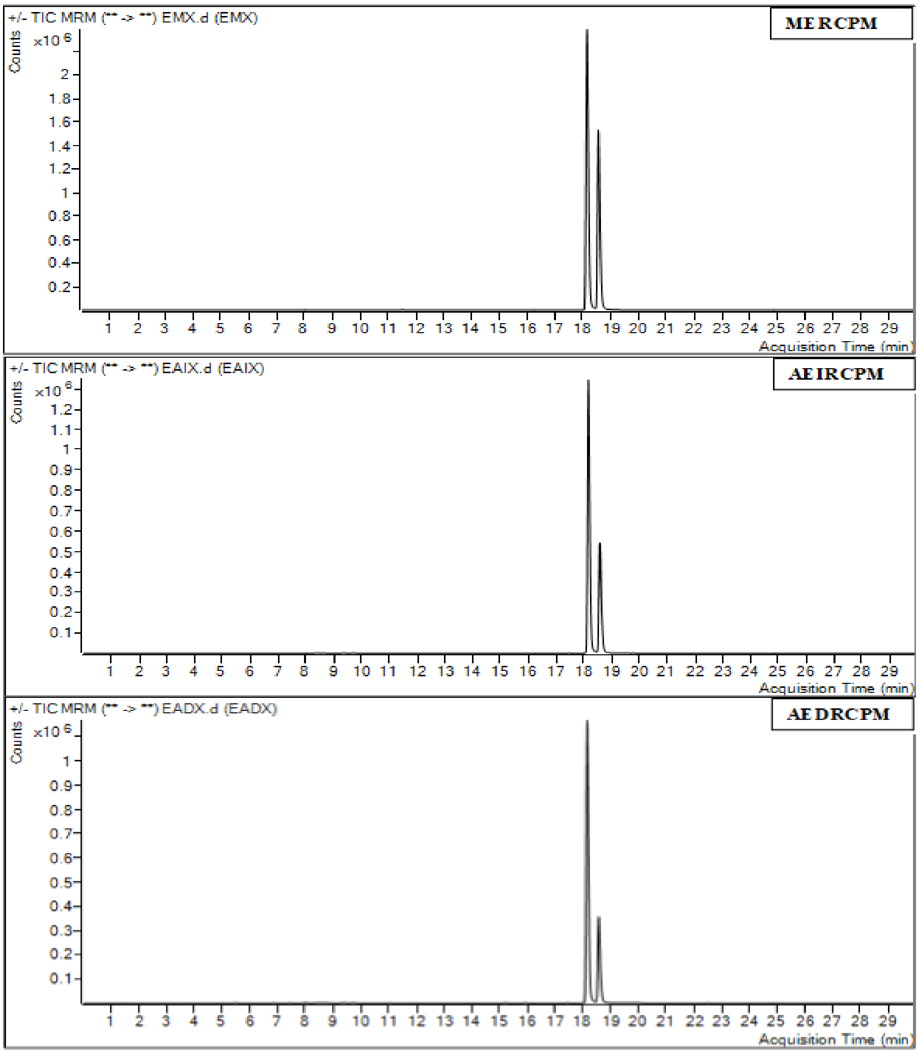

Figure 1. Chromatographic profiles obtained by LC-MS analysis for the different phenolic extracts of the different *C. annum* varieties mixtures.

Other minor components were also identified and quantified, including naringenin, chrysin, baicalin, fisetin, kaempferol-3-glucoside, quercetin-3-glucoside, protocatechuic ethyl ester, *p*-coumaric acid, scutellarin, sinapic acid, salicylic acid, *o*-coumaric acid, vanillin, and protocatechuic acid in the aqueous extract labeled AEDRCPM (Table 4). In addition to these compounds, caffeine was also detected and quantified in the aqueous extract labeled AEIRCPM (Table 3). In the methanolic extract, all of the aforementioned compounds were identified along with additional constituents such as isoquercitrin (49.0512 µg/mL), hesperidin (2.7533 µg/mL), and *o*-coumaric acid (14.3396 µg/mL). Overall, LC-MS analysis revealed a wide range of bioactive compounds in red chili pepper extracts, with catechin, capsaicin, and dihydrocapsaicin being the major components.Hudáková et al. (2023) reported capsaicin contents ranging from 2.1 to 124.2 mg/g and dihydrocapsaicin from 5.1 to 151.3 mg/g in peppers, and demonstrated that higher phenolic contents were associated with stronger antioxidant activity. Similarly, Ornelas-Paz et al. (2010) reported capsaicin levels between 0.6 and 913.8 µg/g, dihydrocapsaicin at 756.9 µg/g, and nordihydrocapsaicin at 68.2 µg/g, with total phenolics ranging from 1150.5 to 2190.0 µg GAE/g in raw peppers, depending on the variety. In a recent study, Ahmed et al. (2022) identified seventy-one chemical compounds belonging to different classes, including alcohols (26.13%), hydrocarbons (>18.82%), esters (>14.97%), ketones (>3.08%), acids (>1.07%), sugars (>0.72%), aldehydes (>0.42%), and amino compounds (>0.15%), with alcohols and hydrocarbons being the most abundant in *Capsicum* fruit samples. Based on the LC–MS data, the rich phytochemical composition of these extracts suggests that dietary intake of such spices may contribute to beneficial health effects.

Table 2. Phenolic compounds identified in MERCPM extract

No.	Compounds	RT (min)	Final conc. (µg/mL)	No.	Compounds	RT (min)	Final conc. (µg/mL)
1	Catechin	6.904	779.0452	12	Isoquercitrin	11.687	49.0512
2	Syringic acid	8.398	184.5894	13	Quarcetin-3-glucoside	12.512	159.3812
3	Vanillin	8.615	13.0995	14	Kaempferol-3-glucoside	12.996	4.8446
4	o-Coumaric acid	9.409	14.3396	15	Fisetin	13.327	7.6175
5	Salicylic Acid	9.707	125.3260	16	Baicalin	13.822	2.5089
6	*trans*-ferulic acid	10.174	20.8749	17	Chrysin	14.221	0.1333
7	Sinapic acid	10.439	18.6212	18	Quercetin	14.619	226.4905
8	Scutellarin	11.164	0.2081	19	Naringenin	14.763	51.8389
9	p-coumaric acid	11.497	6.4864	20	Capsaicin	18.152	701.6327
10	Protocatechuicethyl ester	11.637	5.7871	21	Dihydrocapsaicin	18.565	563.5075
11	Hesperidin	11.687	2.7533				

Table 3. Phenolic compounds identified in AEIRCPM extract

No.	Compounds	RT (min)	Final conc. (µg/mL)	No.	Compounds	RT (min)	Final conc. (µg/mL)
1	Syringic acid	8.383	229.3785	10	Protocatechuicethyl ester	11.598	5.6538
2	Caffein	8.396	16.1512	11	Quarcetin-3-glucoside	12.394	2.5027
3	Vanillin	8.631	31.9287	12	Kaempferol-3-glucoside	13.021	3.9950
4	o-Coumaric acid	9.354	24.8503	13	Fisetin	13.327	7.6109
5	Salicylic Acid	9.699	120.5031	14	Baicalin	13.696	2.4866
6	*trans*-Ferulic acid	10.174	42.6298	15	Chrysin	14.112	0.1327
7	Sinapic acid	10.424	19.2286	16	Naringenin	14.814	75.3163
8	Scutellarin	11.195	0.1992	17	Capsaicin	18.161	374.3950
9	p-Coumaric acid	11.474	6.9472	18	Dihydrocapsaicin	18.581	193.5380

Table 4. Phenolic compounds identified AEDRCPM extract

No.	Compounds	RT (min)	Final conc. (µg/mL)	No.	Compounds	RT (min)	Final conc. (µg/mL)
1	Protocatechuic acid	5.514	36.3417	11	Protocatechuicethyl ester	11.590	5.6550
2	Vanillic acid	7.782	930.6238	12	Quarcetin-3-glucoside	12.504	2.3154
3	Syringic acid	8.359	187.0210	13	Kaempferol-3-glucoside	13.030	5.3700
4	Vanillin	8.631	31.9375	14	Fisetin	13.234	7.6136
5	o-coumaric acid	9.401	16.1699	15	Baicalin	13.847	2.5008
6	Salicylic Acid	9.433	55.2833	16	Chrysin	14.204	0.1357
7	*trans*-Ferulic acid	10.064	129.7444	17	Naringenin	14.797	58.4949
8	Sinapic acid	10.432	19.3942	18	Capsaicin	18.152	328.1265
9	Scutellarin	11.203	0.2748	19	Dihydrocapsaicin	18.573	124.4608
10	p-Coumaric acid	11.529	7.3827				

3.3. Antioxidant Activity

3.3.1. DPPH Free Radical Scavenging

Results of the DPPH free radical scavenging activity are presented in Figure 2, while the corresponding IC$_{50}$ and IC$_{100}$ values (mg/mL) are summarized in Table 5. The mixture of different *C. annuum* extracts exhibited notable antiradical activity, with the highest DPPH inhibition (59.65% ± 0.39) observed at 80 mg/mL for AEDRCPM. Among the tested extracts, the decoction extract (AEDRCPM) showed the strongest activity, as indicated by the lowest IC$_{50}$ value (2.09 mg/mL). In contrast, the other extracts demonstrated lower DPPH scavenging activity, with inhibition percentages below 50%. Nevertheless, measurable activity was observed even at moderate concentrations, with inhibition values of 37.33% ± 0.84 and 21.97% ± 2.23 at 35 mg/mL for MERCPM and AEIRCPM, respectively. However, the antioxidant activity of all extracts remained lower than that of the standard, ascorbic acid. At 35 mg/mL, ascorbic acid exhibited a DPPH inhibition of 51.22% ± 3.32, which increased to 81.45% ± 0.039 at 80 mg/mL. The IC_{50} and IC_{100} values for ascorbic acid were 3.22 mg/mL and 5.77 mg/mL, respectively. Overall, AEDRCPM and MERCPM can be considered the most active extracts, demonstrating relatively strong antioxidant potential, with IC_{100} values of 3.84 mg/mL and 4.96 mg/mL, respectively.

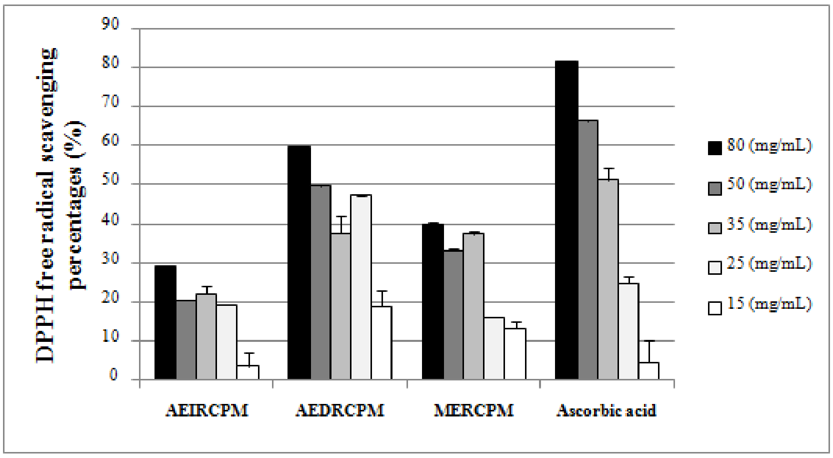

Figure 2. DPPH free radical scavenging activity (%) of extracts from the mixture of different red chili pepper varieties

Table 5. Inhibitory concentrations 50 (IC_{50}) and inhibitory concentrations 100 (IC_{100}) of the various red chili pepper extracts

Samples / Concentrations (mg/mL)	**IC_{50}**	**IC_{100}**
MERCPM	2.6	4.96
AEIRCPM	3.97	7.16
AEDRCPM	2.09	3.84
Ascorbic acid	3.22	5.77

The evaluation of the antioxidant activity of the various red chili pepper extracts allowed us to qualify these extracts as potent antioxidants with higher efficacy in neutralizing the DPPH free radicals, with higher inhibition percentages exceeding 50%. Grojja et al. (2023) demonstrated that *C. annuum* extracts collected from Tunisia have a strong anti-radical effect, with an IC_{50} value of 45.0 µg/mL. Thus, Ivan et al. (2024) confirmed the antioxidant potential of red pepper phenolic extracts with an IC_{50} concentration of 1.669 mg/mL. These results confirmed the red pepper richness in more powerful antioxidant molecules, which motivates their use as food supplement in our diet in order to enrich the body with antioxidants and to maintain an oxidant-antioxidant balance.

3.3.2. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was used to evaluate the antioxidant capacity of the red chili pepper mixture extracts based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The results indicated that all phenolic extracts exhibited considerable ferric reducing power, comparable to that of the standard (ascorbic acid). Higher absorbance values were observed for the extracts, reflecting stronger reducing capacity compared to the standard (Figure 3). Among the tested samples, the extracts labeled AEDRCPM and AEIRCPM demonstrated the highest ferric reducing antioxidant activity, indicating their potential as effective reducing agents.

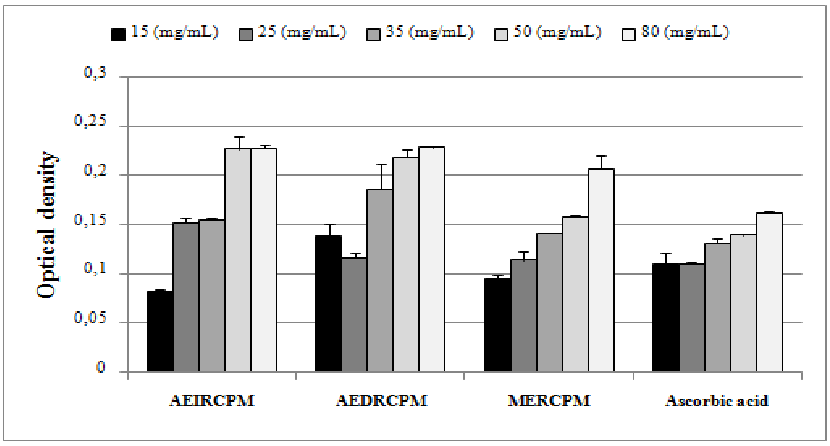

Figure 3. Ferric reducing antioxidant power of the different red chili pepper extracts

The aqueous extract prepared by infusion (AEIRCPM) exhibited the highest ferric reducing antioxidant capacity, with the greatest content of antioxidant compounds (26.25 ± 0.68 mg AAE/g AEIRCPM). This was followed by the methanolic extract (MERCPM) and the decoction extract (AEDRCPM), with total antioxidant contents of 12.88 ± 2.45 mg AAE/g and 9.13 ± 0.13 mg AAE/g, respectively (Figure 4). The use of a mixture of different *C. annuum* varieties for phenolic extraction appears to be an effective strategy to enhance phytochemical diversity, antioxidant potential, anti-inflammatory activity, and overall functional value. Farajzadeh Memari Tabrizi et al. (2025) similarly reported that methanolic extracts of *C. annuum* possess strong antioxidant capacity, highlighting their potential role in mitigating oxidative stress associated with chronic diseases such as cardiovascular disorders, neurodegenerative conditions, and cancer. The antioxidant activity observed in this study can be attributed to the presence of various bioactive compounds, including capsaicin, dihydrocapsaicin, quercetin, isoquercitrin, syringic acid, salicylic acid, trans-ferulic acid, naringenin, chrysin, baicalin, fisetin, kaempferol-3-glucoside, protocatechuic ethyl ester, *o*-coumaric acid, *p*-coumaric acid, scutellarin, sinapic acid, vanillin, protocatechuic acid, and hesperidin identified in the phenolic extracts of the mixed varieties.

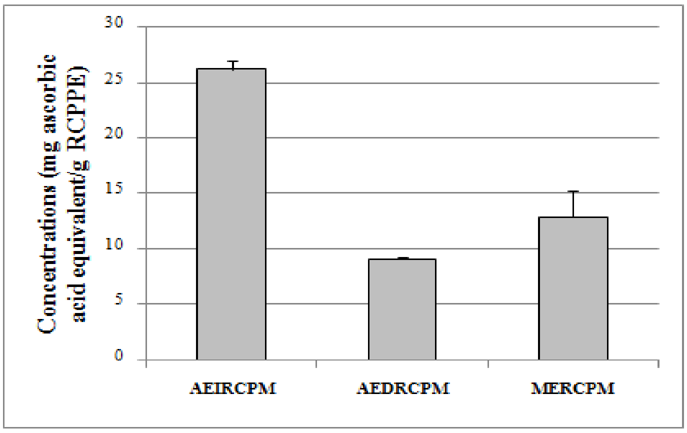

Figure 4. Concentrations are expressed as milligrams of ascorbic acid equivalents per gram of red chili pepper phenolic extracts (mg AAE/g RCPPE) for ferric reducing antioxidant capacity

3.4. In-vitro Anti-Inflammatory Activity

The inhibition of bovine albumin denaturation is presented in Figure 5. All red chili pepper extracts exhibited strong anti-inflammatory activity, with inhibition percentages exceeding 80% at higher concentrations. The highest inhibition was observed at 80 mg/mL, reaching 97.25 ± 0.30%, 96.58 ± 0.24%, and 94.82 ± 0.03% for MERCPM, AEIRCPM, and AEDRCPM, respectively. In contrast, the standard drug, diclofenac sodium, showed a lower inhibition percentage (48.56 ± 0.12%) under the same conditions. These findings indicate that the mixture of different *C. annuum* varieties used for phenolic extract preparation exhibits remarkable anti-inflammatory potential, particularly in inhibiting protein denaturation.

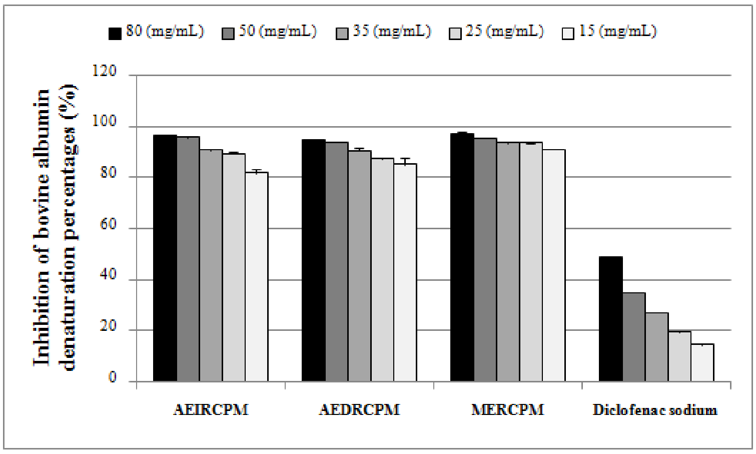

Figure 5. Effect of different red chili pepper varieties mixture used in phenolic extracts preparation on the inhibition of bovine albumin denaturation

Overall, the anti-inflammatory evaluation indicated that the red chili pepper extracts prepared in this study were highly effective in inhibiting BSA denaturation. A recent study by Touzouirt et al. (2025) on Algerian red chili pepper extracts reported significant anti-inflammatory activity in an animal model, with edema reduction of 75.13% at 0.13 g/mL and up to 85% using a topical ointment formulation. Similarly, Tipduangta et al. (2025) demonstrated that red chili pepper extracts exhibit strong antioxidant activity along with comparable anti-inflammatory effects. The use of mixed varieties of *C. annuum* enhances the diversity and abundance of phenolic compounds, including capsaicin, dihydrocapsaicin, catechin, quercetin, quercetin-3-glucoside, syringic acid, salicylic acid, vanillic acid, trans-ferulic acid, naringenin, chrysin, baicalin, fisetin, kaempferol-3-glucoside, protocatechuic ethyl ester, *o*-coumaric acid, *p*-coumaric acid, scutellarin, sinapic acid, vanillin, protocatechuic acid, caffeine, isoquercitrin, and hesperidin. These compounds collectively contribute to the antioxidant and anti-inflammatory activities observed, thereby supporting their potential role in preventing diseases associated with oxidative stress and inflammation.

4. CONCLUSION

The growing demand for natural compounds with therapeutic potential has intensified interest in plant-based bioactive sources. In this context, the present study explored the potential of different Algerian red chili pepper varieties as sources of antioxidant and anti-inflammatory agents. A mixture of four *C. annuum* varieties Jalapeño, Baklouti, Black Prince, and Black Cobra was employed to enhance the bioactivity of both methanolic and aqueous extracts. The results demonstrated strong antioxidant activity, with high DPPH radical scavenging effects and significant ferric reducing power, particularly for the aqueous extract obtained by infusion (AEIRCPM). The combined use of multiple varieties contributed to enhanced bioactivity compared to individual extracts, likely due to the synergistic interactions among the identified phenolic compounds. LC-MS analysis confirmed the presence of key bioactive constituents, including capsaicin, dihydrocapsaicin, and various flavonoids and phenolic acids, which collectively contribute to free radical scavenging. In addition, all extracts exhibited notable anti-inflammatory activity, with bovine albumin denaturation inhibition exceeding 80% and surpassing the effect of diclofenac. These findings highlight the potential of Algerian red chili pepper cultivars as rich sources of bioactive compounds with significant antioxidant and anti-inflammatory properties, supporting their potential application in the prevention of oxidative stress-related and inflammatory disorders.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTION
Nour El Houda Bekkar and Foufa Bouabsa: Conceptualization, Methodology, Software. Nour El Houda Bekkar, Foufa Bouabsa, Boumediene Meddah, Farah Gaidi, Bouchra Gaidi, Ilyas Yildiz and Erenler Ramazan: Data collection, Writing original draft. Nour El Houda Bekkar: Visualization, Investigation. Nour El Houda Bekkar and Walid Hassene Hamri: Supervision. Nour El Houda Bekkar and Foufa Bouabsa: Software, Validation. Nour El Houda Bekkar and Walid Hassene Hamri: Writing, Reviewing and Editing.

DATA AVAILABILITY
All data generated or analyzed during this study are included in this published article.

DECLARATION OF GENERATIVE AI
Not applicable.

ETHICS
Not applicable.

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