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RESEARCH

Phytochemical content, antioxidant, and anti-inflammatory activities of Morrocan *Cynara cardunculus* L. var. *ferocissima* leaf methanolic extract

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Abstract

Cyanra cardunclus L. var. *ferocissima* is widely used in Morocco as a food and in traditional medicine. Therefore, this study aimed to determine, for the first time, the phytochemical content and antioxidant and anti-inflammatory activities of this variety. Qualitative tests were used to screen for the phytochemical compounds present in the extract, and spectrophotometric methods were used for quantification. The sugar profiles were determined using HPLC. Antioxidant activity was determined in vitro using DPPH, FRAP, and total antioxidant activity assays, and anti-inflammatory activity was assessed using serum albumin denaturation and membrane stabilization assays. The extract contained a high amount of total polyphenols, hydrocinnamic acids, anthocyanins, chlorophyll, ortho-diphenols, terpenoids, and triterpenoids. In addition, five sugars were identified with high amounts of raffinose and sucrose. The extract exerted considerable antioxidant activity by scavenging radicals and reducing power. It exerts anti-inflammatory effects by inhibiting protein denaturation and heat-inducing hemolysis. From the correlation results, anthocyanin, polyphenol, and triterpenoid contents were strongly correlated with DPPH free radical scavenging activity. Orthodiphenols, flavonols, and chlorophyll α were strongly correlated with FRAP, whereas orthodiphenols, hydrocinnamic acids, and triterpenoids were strongly correlated with total antioxidant activity. In terms of anti-inflammatory activity, orthodiphenols, hydrocinnamic acids, and triterpenoids correlated strongly with inhibition of bovine serum albumin denaturation activity, whereas terpenoids, flavonols, and chlorophyll correlated strongly with red cell membrane-stabilizing activity. In conclusion, the Moroccan *Cynara cardunclus var. ferocissima* leaf methanolic extract constitutes a promising source of phytochemicals with considerable antioxidant and anti-inflammatory activity.

Keywords *Cyanra cardunclus* L. var. *Ferocissima* · Phytochemicals · Antioxidant · Anti-inflammatory · Correlation analysis

Abbreviations

AAE	Ascorbic acid equivalent
BSA	Bovine serum albumin
DW	Dry weight
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FRAP	Ferric-reducing power
GAE	Gallic acid equivalents
HPLC	High-performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
QE	Quercetin equivalent
RUE	Rutin equivalent
TAC	Total antioxidant capacity
TPTZ	2, 4, 6-Tris (2-pyridyl)-s-triazine
UAE	Ursolic acid equivalent

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Introduction

The Asteraceae family is a large family of flowering plants comprising over 25,000 species worldwide. This family includes plants with great medicinal value, endowed with antioxidant, anti-inflammatory, and antimicrobial properties [1]. *Cynara cardunculus*, belonging to the Asteraceae family (Compositae), genus Cynara, is widespread in the Mediterranean region. It has a high nutritional value, making it an interesting ingredient in the human diet [2]. The leaves of *C. cardunculus* have demonstrated promising pharmacological properties in in vitro and in vivo studies. It has antioxidant, anti-inflammatory, hypolipidimiant, hypoglycemic, hepatoprotective, and anti-atherosclerotic effects [3]. Various phytochemical compounds have been reported in the leaves of *C. cardunculus*. Phenolic compounds are mainly represented by phenolic acids, especially chlorogenic acid and dicaffeoylquinic acid derivatives, and flavonoids, especially luteolin, apigenin, and their derivatives. They also contain sesquiterpene lactones, with an abundance of cynaropicrin and grosheimin, and pentacyclic triterpenes [4].

Cynara cardunclus L. var. *ferocissima*, also known as Madeira cardoon, is characterized by its blue flowers, which resemble artichokes [5]. The leaf rachises of *C. ferocissima* are commonly used in human diets in the preparation of many dishes, and the flowers are traditionally used in the manufacture of some types of cheeses [5]. Little scientific interest has been directed at phytochemical studies and the biological activities of the *C. ferocissima* variety. The aim of this study was to determine the phytochemical content of this plant growing in Morocco and analyze its antioxidant and anti-inflammatory activities.

Materials and Methods

For the material and methods, the data are presented as Online Resource 1.

Results and Discussion

Medicinal plants are a source of bioactive compounds, among which polyphenols and terpenoids are the most widely distributed. Therefore, the first part of our work focused on determining the nature of the compounds present in the methanolic extract of *C. ferocissima* using qualitative tests and determining the quantity of phytochemical compounds using spectrophotometric methods. The second part focused on evaluating its antioxidant and anti-inflammatory activities using in vitro tests and correlating the phytochemical content with these biological activities.

Extraction Yield

The *C. ferocissima* leaves were extracted by maceration using aqueous methanol. A high extraction yield was obtained (34.16 ± 4.62%) (Table 2). The yield obtained is higher than the yield of ethanolic (16.37 ± 0.46%) and aqueous (29.40 ± 1.47%) extracts of Moroccan cardoon leaves obtained by soxhlet and ultrasound (24.00 ± 2.36% for the ethanolic extract and 33.00 ± 4.98% for the aqueous extract) [6].

Qualitative Analysis

Preliminary phytochemical screening is required to determine the nature of the bioactive compounds present in plants. The results show that the methanolic extracts of the *C. ferocissima* leaves contained phytochemicals of different classes, including phenols, flavonoids, tannins, particularly catechin tannins and phlobatannins, lignins, steroids, terpenoids, triterpenoids, cardiac glycosides, quinones, and volatile oils (Table 1). These findings are in agreement with other studies demonstrating the presence of a wide range of phytochemicals in the leaves of Cynara species, such as *C. scolymus*, which contains cardiac glycosides, triterpenoids, saponins, flavonoids, and tannins [7].

Quantitative Phytochemical Analysis

The total phenolic content of the methanolic extract was determined using the Folin-Ciocalteu method. As shown in Table 2, the methanolic extract of *C. ferocissima* leaves has a high total polyphenol content of 157.77 ± 11,82 mg gallic acid equivalent *per* gram of extract (mg GAE/g extract), which was higher than that reported in another study conducted in Ponta de São Lourenço (185 ± 3 mg GAE/100 g dry plant) [8]. The total hydroxycinnamic acid content in the extract was 127.94 ± 17,6 mg GAE/g extract (Table 2). As reported in previous studies, caffeoylquinic acid isomers are the major phenolic acids present in this variety.

Table 1 Phytochemical screening of a methanolic extract of *C. Ferocissima*

Compounds	Ph	Fl	CT	HT	PT	Co	Li	Sr	Tr	Ttr	Ir	CG	Qu	VO
Test result	+	+	+	-	+	+	+	+	+	+	-	+	+	+

Ph – Phenolic compounds, Fl – flavonoids, CT – Catechic tannin, HT – Hydrolysable tannin, PT – Phlobatannin, Co – Coumarins, Li – Lignin, Sr – Steroïds, Tr – Terpenoïds, Ttr–Triterpenoïds, Ir –Iridoids, CG – Cardiac glycoside, Qu – Quinones, VO – Volatile oil. (+) means detected while, (–) means not detected

Table 2 Extraction yield and phytochemical content of *C. Ferocissima* leaf methanolic extract

Parameters	Result
Extraction yield (%)	34. 16 ± 4.62
Total polyphenol content (mg GAE/ g extract)	157.77 ± 11,82
Total flavonoid content (mg QE/ g extract)	34.29 ± 0,19
Total flavonol content (mg QE/ g extract)	27.20 ± 0,73
Total phenolic acid content (mg GAE/g extract)	127.94 ± 17,6
Total anthocyanidin content (µg cyanidin-3-glucoside/g DW)	38.84 ± 1,54
Total ortho-diphenol content (mg GAE/ g extract)	877.07 ± 9.31
Total terpenoids content (mg UAE/ g extract)	3558.07 ± 0.13
Total triterpenoïds content (mg UAE/g extract)	3313,72 ± 0.44
Chlorophyll α (µg/ml)	4.67± 0.087
Chlorophyll ß (µg/ml)	10.69 ± 1.12

Values are expressed as mean ± SD ($n = 3$). GAE – gallic acid equivalents, QE – quercetin equivalent, UAE – ursolic acid equivalent

The levels of caffeoylquinic acid quantified in the other geographical areas were 253 ± 0.94 mg/100 g DW [8] and 241 ± 1 mg/100 g DW [9], which indicated that the Moroccan extract had higher values. Moreover, the total flavonoid content in the extract was 34.29 ± 0,19 mg quercetin equivalent *per* g of extract (QE/g extract), which was higher than that obtained in Madeira Island (2.86 ± 0.0010 mg RUE/100 g plant material) [8]. The flavonol content in the extract was 27.20 ± 0,73 mg QE/g of extract, which indicates that more than 60% of the flavonoids in the extract are flavonols. Furthermore, the extract contained an important amount of anthocyanins (38.84 ± 1,54 µg cyanidin-3-glucoside/g DW), higher to that reported in leaves of the *Blanc d'Oran* variety of Tunisian artichoke (20.5 ± 1.4 µg cyanidin-3-glucoside/g DW) [10]. In addition, the leaf extract contained higher amounts of chlorophyll α (4.67 ± 0.087 µg/mL), which is lower than that reported in artichoke leaves (9.8 ± 0.89 µg/mL), and high amounts of chlorophyll ß (10.69 ± 1.12 µg/mL), which was higher than that of the artichoke leaf ethanolic extract (6.0 ± 0.17 µg/mL) [10].

Among polyphenols, ortho-diphenol compounds are powerful antioxidants because of their ability to form intramolecular hydrogen bonds, stabilizing the phenoxyl radical formed after radical scavenging. Cynara species such as *C. scolymus* are rich in ortho-diphenol compounds (chlorogenic acid, cynaroside, scolymoside, and cynarin) [11]. The methanolic extract of *C. ferocissima* leaves contains a high ortho-diphenol amount of 877.07 ± 9.31 mg GAE/g extract, superior to the level in Tunisian artichoke leaf ethanolic extract (58.8 ± 2.0 mg/g DW) [10].

On the other hand, terpenoids are a diverse family of compounds with promising antioxidant, antibacterial, anti-inflammatory, and anticancer activity. The leaves of Cynara species such as *C. cardunculus* var. *altilis* are rich in terpenoids, particularly sesquiterpene lactone, cynaropicrin, grosheimin, and pentacyclic triterpenes, particularly β-amyrin, lupeol, ψ-taraxasterol, and taraxasterol [12]. Artichoke leaves are also rich in sesquiterpenes, particularly cynaropicrin, grosheimin, and cynaratriol [13]. This study is the first to quantify terpenoids and triterpenoids in the leaves of this variety. The amount of total terpenoids in the extract was 3558.07 ± 0.13 mg of ursolic acid equivalent *per* gram of extract. The majority of terpenoids in the extract were triterpenoid, which represents 3313.72 ± 0.44 mg of ursolic acid *per* gram of extract.

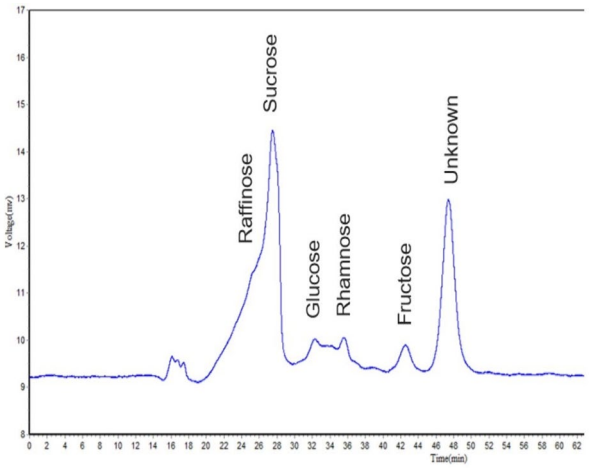

Fig. 1 Chromatogram of carbohydrate composition of *Cynara cardunculus* L. var. *ferocissima* leaf methanolic extract

Determination of Carbohydrate Profile by HPLC Analysis

HPLC was used to determine the sugar profile of the *C. ferocissima* methanolic extract. The results obtained from HPLC chromatograms revealed the presence of five sugars: sucrose, glucose, rhamnose, and fructose (Fig. 1). Sucrose accounted for almost half of the sugars (43%) in the extract, followed by raffinose (24%). Glucose, rhamnose, and fructose were present in low quantities (Table 3). An unknown peak at a retention time of 47.0 min was also detected, possibly a cyclitol. The types of sugars identified in this extract

Table 3 The content of carbohydrate compounds of *C. Ferocissima* leaves methanolic extract determined by HPLC

Compound	Retention time (min)	Percentage amount (%)
Raffinose	25.8	24.2
Sucrose	27.5	42.8
Glucose	32.1	1.2
Rhamnose	35.8	1.2
Fructose	42.5	3.8
Unknown	47.0	26.8

Table 4 The antioxidant activity of *Cyanra cardunclus* L. var. *Ferocissima* leaves methanolic extract determined by DPPH, FRAP, and TAC assays

Assay	Unit	Extract	Ascorbic acid
DPPH	IC_{50} (mg/mL)	0.248 ± 0.008^{a}	$0.093 \pm 0{,}014^{b}$
FRAP	mg AAE/g extract	104.01 ± 0.429	-
TAC	mg AAE/g extract	176.51 ± 1.56	-

Values are expressed as mean ± SD ($n = 3$), EAA – ascorbic acid equivalent, mean values within a line with different letters are significantly different at $p < 0.05$

were similar to those of methanol leaf extracts from Egyptian artichokes, which contained sucrose, glucose, fructose, and rhamnose with an abundance of sucrose [14].

Antioxidant Activity

Phytochemicals inhibit oxidative stress by inhibiting free radicals through hydrogen and electron donation or by metal chelation. To assess the antioxidant activity of the methanolic extract of *C. ferocissima*, DPPH, FRAP, and TAC tests were carried out, and the results are presented in Table 4. The results show that the leaf extract of *C. ferocissima* was effective in scavenging DPPH radicals in a dose-dependent manner, as shown by the increase in the percentage of DPPH radical inhibition with increasing extract concentration. The results indicated that the methanolic leaf extract had an inhibitory concentration of $0{,}248 \pm 0.008$ mg/mL, which remained higher than that of ascorbic acid ($0.093 \pm 0{,}014$ mg/mL) ($p < 0.05$), demonstrating that the antioxidant activity of the extract was significantly lower than that of the reference antioxidants. In comparison with the antioxidant activity of other Cynara species, the antioxidant activity of our extract is higher than that of the methanolic extract of Moroccan cardoon leaves, with IC_{50} values of 1.11 mg/mL for the ethanolic extract and 2.07 mg/mL for the water extract [6]. Moreover, the *C. ferocissima* leaf extract has a 176.51 ± 1.56 mg ascorbic acid equivalent *per* gram of extract (AAE/g extract) total antioxidant capacity, which remains higher than that of the ethanolic extract of Moroccan *Cynara humulis* leaves (0.77 ± 0.07 mg AAE/g extract) [15]. It is also higher than the total antioxidant activity of the ethanolic extract (84.76 ± 3.83 mg AEE/g fresh matter) of Moroccan cardoon leaves [6]. Regarding

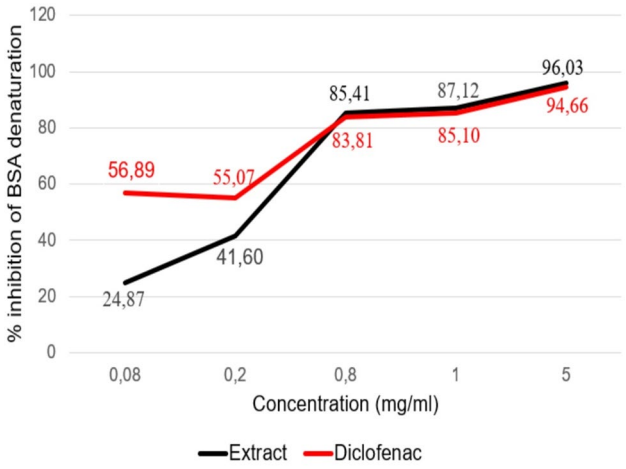

Fig. 2 Percentage inhibition of BSA denaturation according to the concentration of extract and diclofenac sodium

Table 5 Inhibition activity of BSA denaturation in varying concentrations of extract and sodium diclofenac

Concentration (mg/mL)	Extract	Sodium diclofenac
0,08	24.87 ± 7.65^{a}	56.89 ± 1.53^{b}
0,2	41.60 ± 2.19^{a}	55.07 ± 4.52^{b}
0,8	85.41 ± 0.80^{a}	83.81 ± 0.54^{b}
1	87.12 ± 0.91^{a}	85.10 ± 1.62^{a}
5	96.03 ± 6.08^{a}	94.66 ± 0.22^{a}
IC_{50} (mg/mL)	0.390 ± 0.004^{a}	0.419 ± 0.018^{a}

Values are represented by the mean of three replicates ± SD. Mean values within a line with different letters are significantly different at ($p < 0.05$)

the FRAP assay, the extract has an important reducing power of 104.01 ± 0.42 mg AAE/g extract, demonstrating the presence of ferric-reducing compounds in the extract.

Determination of the anti-inflammatory Activity

Denatured proteins are pro-inflammatory and trigger the inflammation process, leading to serious sequelae such as rheumatoid arthritis, Alzheimer's disease, and atherosclerosis. The inhibition of protein denaturation assay was evaluated using bovine serum albumin as a substrate by testing different extract concentrations. The results showed that the protein denaturation inhibitory activity of the extract increased with increasing extract concentration (Fig. 2). The activity of the extract is similar to that of diclofenac, as demonstrated by the IC_{50} of the extract (0.39 ± 0.004 mg/mL), which is not significantly different ($p = 0.061$) from that of sodium diclofenac (the $IC_{50} = 0.41 \pm 0.01$ mg/mL). In comparison with another Cynara species, the anti-inflammatory activity of the Cynara leaf methanolic extract, which inhibits $87.12 \pm 0.91\%$ of BSA denaturation at a concentration of 1 mg/ml (Table 5), is higher than that of the *Cynara scolymus* L. flower stem methanolic extract, which inhibits

Table 6 In vitro anti-inflammatory activity of *C. Ferocissima* leaf methanolic extract

Concentration (mg/mL)	Inhibition of heat induced hemolysis (%)		Inhibition of hypotonicity induced hemolysis (%)	
	Extract	Indomethacin	Hypotonicity	Indomethacin
0.08	28.32± 1.05[a]	28.8± 2.52[a]	8.47± 0.89[a]	10.2 ± 0,70[a]
0.2	33.12± 1.91[a]	35.44± 1.45[a]	9.5 ± 1.92[a]	19.1± 3,18[b]
0.4	39.15± 1.43[a]	38.82± 5.72[a]	15.38 ± 1,24[a]	24.8 ± 3.47[b]
0.8	40.16± 0.63[a]	39.76±0.25	34.06 ± 4,36[a]	57.2± 4.77[b]
2	45.58± 3.10[a]	40.49± 1.80[a]	84.68 ± 2,41[a]	99.05± 1.93[b]
IC_{50}	2,39± 0,413[a]	2.81±1.17[a]	1.173 ± 0,02[a]	0.84± 0,02[b]

The values are expressed as means ± S.D ($n = 3$); Mean values within a line with different letters are significantly different at ($p < 0.05$)

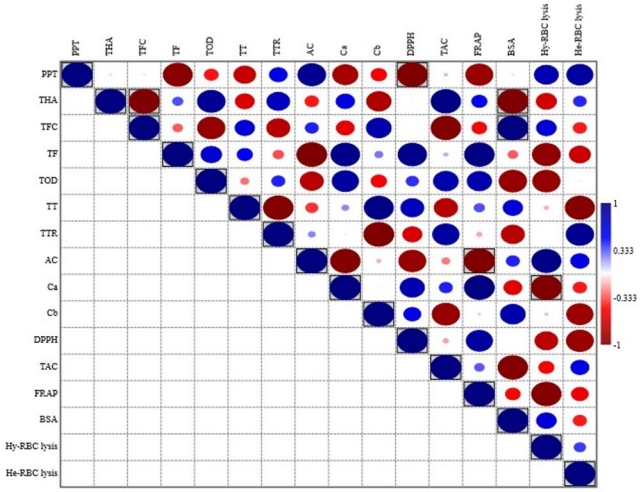

Fig. 3 Correlation between phytochemical content, antioxidant, and anti-inflammatory activities. PPT – total polyphenol, THA – total hydrocinnamic acid, TFC – total flavonoid content, TF – total flavonol, TOD – total orthodiphenols, TT– total terpenoids, TTR – total triterpenoids content, AC – anthocyanins, Ca-chlorophyll-a, Cb – Chlorophyll-b, TAC – total antioxidant activity, DPPH–2, 2-diphenyl-1-picrylhydrazyl, FRAP – ferric-reducing power, BSA – anti bovine serum albumin denaturation, Hy-RBC lysis – hypotonicity red blood cell lysis inhibition, He-RBC lysis – heat red blood cell lysis inhibition

69.54 ± 1.77% of BSA denaturation at some concentration [16].

Lysis of the lysosomal membrane is an essential mechanism in inflammation. Assays based on cell lysis inhibition are commonly used to study the anti-inflammatory activity of drugs in vitro. The extract prevented heat- and hypotonicity-induced hemolysis in a concentration-dependent manner (Table 5). For the hypotonicity-induced hemolysis assay, the extract inhibits lysis of 84.68 ± 2.41% at a concentration of 2 mg/mL; indomethacin inhibits 99.05 ± 1.93% of hypotonic lysis at the same concentration with a significant difference ($p < 0.05$). The inhibitory activity of the extract ($IC_{50} = 1.17 \pm 0.02$) was significantly lower than that of indomethacin ($IC_{50} = 0.84 \pm 0.02$) ($p < 0.05$). In the heat-induced hemolysis inhibition assay, the extract inhibited 45.58 ± 3.10% hemolysis at a concentration of 2 mg/mL, with no significant difference from indomethacin, which inhibited 40.49 ± 1.80% hemolysis. The activity of hemolysis inhibition in the extract ($IC_{50} = 2.49 \pm 0.41$ mg/mL) did not differ significantly from that of indomethacin ($IC_{50} = 2.81 \pm 1.17$ mg/mL), with $p = 0.078$. The significant hemolysis-inhibiting activity of the methanolic extract of *C. ferocissima* confirms the important red blood cell membrane-stabilizing activity of Cynara species, specifically Cynara scolymus leaves [17] (See Table 6).

Correlation between Bioactive Compound Content and Biological Activities

Pearson's correlation coefficient was calculated to predict the phytochemical compounds responsible for the antioxidant and anti-inflammatory activities of the extract (Fig. 3). A strong negative correlation was observed between DPPH and total polyphenol (r = -0.99), anthocyanins (r = -0.89), and triterpenoids (r = -0.63), which demonstrate the potential of these compounds for DPPH radical scavenging activity. In addition, a strong positive correlation was observed between FRAP and orthodiphenols ($r = 0.80$); a very strong correlation with total flavonols ($r = 0.98$); chlorophyll α ($r = 0.99$); and a very strong negative correlation with total polyphenols (r = -0.89) and anthocyanins (r = -0.99). The positive coefficient of FRAP indicates that flavonols, orthodiphenols, and chlorophyll are strongly involved in ferric-reducing power. In addition, total hydroxycinnamic acid ($r = 0.97$), triterpenoids ($r = 0.87$), and orthodiphenols ($r = 0.82$) showed a very strong correlation with total antioxidant capacity.

In addition, hydrocinnamic acids, orthodiphenols, and triterpenoids are associated with the anti-inflammatory activity of the extract by inhibiting BSA denaturation. This result is explained by the very strong negative correlation with hydrocinnamic acids (r = -0.99) and orthodiphenols (r = -0.91) and the strong correlation with triterpenoids (r = -0.77). On the other hand, the terpenoids, flavonols, and chlorophyll were correlated with the anti-inflammatory activity measured by hypotonicity and heat-induced hemolysis assays. The inhibitory activity of hypotonic hemolysis correlates very strongly with chlorophyll a (r = -0.99),

flavonols (r = -0.92), and orthodiphenols (r = -0.90), while the inhibitory activity of heat-induced hemolysis correlates very strongly with terpenoids (r = -0.97) and strongly with chlorophyll b (r = -0.87) and flavonols (r = -0.70).

Conclusion

In conclusion, this research provides an initial report on the phytochemical composition and biological properties of Moroccan *C. ferocissima* leaf extract. As shown, methanolic extract is rich in total polyphenols, phenolic acids, anthocyanin, orthodiphenols, chlorophyll, and terpenoids. These compounds provide the extract an important antioxidant activity by scavenging radicals and reducing ferric ions, as well as anti-inflammatory activity through inhibiting protein denaturation and heat hemolysis. The important biological properties of this plant part encourage its daily consumption as a supplement for treating and preventing diseases caused by oxidative stress and inflammation including neurodegenerative, cancer and cardiovascular diseases. For this purpose, the characterization and the quantification of the individual compounds present in the extract are necessary, and further investigations must be conducted to evaluate its safety and extend the spectrum of other biological activities in vitro and in vivo.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Human and Animal Participants This article does not contain any studies with human or animal subjects.

Competing Interests The authors declare no competing interests.

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