



Phytochemical Characterization, Antioxidant, and Anti-inflammatory Activities of *Chamaerops humilis* L. Pulps Extracts and Tocopherols Content from Two Different Moroccan Regions

Hajar Taibi^{1,2,3}, Aya Khouchlaa^{2,*}, Ahmed Hajib⁴, Hamid Khamar⁵, Fatna Zaakour⁶, Lala Laaziza Ichir³, Abdeslam Meliani¹

¹ Laboratory of Applied Organic Chemistry, Faculty of Sciences and Technology Fez, Morocco; hajartaibi2000@gmail.com (H.T.); meliani_abdeslam@yahoo.fr (A.M.);

² Biochemistry laboratory, National Agency of Medicinal and Aromatic Plants, BP 159 Principal, Taounate, 34000, Morocco; aya.khouchlaa@gmail.com;

³ Laboratory of Plant, Animal and Agro-industry Production, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco

⁴ Higher School of Education and Training (ESEF), Université Ibn Zohr, Agadir, Morocco; hajib.ahmed1@gmail.com;

⁵ University Mohammed V in Rabat, Scientific Institute, Department of Botany and Plant Ecology, B.P. 703, Rabat 10106, Morocco; khamarhamid90@gmail.com;

⁶ Laboratory of Sustainable Agriculture Management (MAD), Team of Biotechnology and Agricultural Production Improvement (BAPA), Higher School of Technology Sidi Bennour (EST), Chouaib Doukkali University, El Jadida, Higher School of Technology, Av. of FAR, Sidi Bennour, P.O. Box 180-24350 Morocco; zaakour-fatna@hotmail.com;

* Correspondence: aya.khouchlaa@gmail.com (A.K.); hajartaibi2000@gmail.com (T.H.);

Scopus Author ID 57191631959

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Abstract: *Chamaerops humilis* L. is a medicinal species extensively used in traditional medicine due to its biological properties. The objective of this study is to enhance the value of this plant species by conducting a phytochemical screening, quantification of phenolic compounds, and evaluation of antioxidant and anti-inflammatory activities of extracts (organic and aqueous) from two varieties of *C. humilis* collected in two regions in Morocco, viz., Khenifra and Beni-Snassen. Phytochemical screening is performed by employing different reactions of coloration and precipitation. The contents of the flavonoid, total polyphenol, and condensed tannins were determined using the aluminum trichloride method, the Folin-Ciocalteu method, and the vanillin-HCl method, respectively. Antioxidant activity was studied using the DPPH and ABTS tests. Anti-inflammatory activity was determined by studying the inhibition of BSA. Tocopherol content and composition were analyzed using High-Performance Liquid Chromatography (HPLC). The results of phytochemical screening of this plant showed the presence of sterols, flavonoids, tannins, and triterpenes and the absence of saponins and anthraquinone glucosides. The conducted assays showed that the aqueous extracts of both varieties were the richest in polyphenols, with a higher content in the variety collected in Beni Snassen than in Khenifra (48.676 ± 0.013 mg GAE/g of extract, 41.80 ± 0.024 mg GAE/g of extract, respectively). However, the quantification of flavonoids and condensed tannins showed that the hexane extracts had higher values compared to the other extracts. The aqueous extract of *C. humilis* (from Beni Snassen) exhibited significant antioxidant activity compared to the other extracts, with an $IC_{50} = 0.54 \pm 0.14$ mg/mL and an $IC_{50} = 0.11 \pm 0.005$ mg/mL using DPPH and ABTS assays, respectively. Regarding anti-inflammatory activity, an inhibition of 50% was observed in a concentration range from 0.3 mg/mL to 0.61 mg/mL. Moreover, the *C. humilis* pulps from Beni Snassen showed slightly higher inhibition of BSA denaturation than the ones from Khenifra. In addition, the lipophilic fraction was found to contain a total tocopherol of 3118.51 ± 3.99 mg/kg in samples collected from Khenifra, while samples collected from Beni-Snassen had a level of 3408.98 ± 3.76 mg/kg. The high content of polyphenols in *C. humilis*

pulps collected from Beni Snassen explains its antioxidant and anti-inflammatory activities. Thus, this plant could be an important source of natural compounds in the prevention of the development of several diseases.

Keywords: *Chamaerops humilis*; screening; antioxidant; quantitative composition; tocopherols; anti-inflammatory.

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1. Introduction

Natural products constitute a highly valuable asset for international pharmaceutical firms engaged in developing novel drugs. Nowadays, a significant portion of these medications, around 35%, are derived from natural sources, such as plants (ca. 25%), animals (ca. 3%), and microbes (ca. 13%) [1]. Due to its geographical location, Morocco enjoys favorable conditions for developing various medicinal herbs [2]. The country boasts around 5200 taxa (species and subspecies) of vascular plants [3], with a large number of medicinal species belonging to the Lamiaceae, Asteraceae, and Arecaceae families [4, 5].

Chamaerops L. is a genus of flowering plants in the palm family Arecaceae [6, 7]. This monospecific genus (only a single species, *C. humilis* L.) owes its name to the Greek chamai, low; rops, bush. The current distribution of this genus is limited to the West Mediterranean basin [8]. It is found along the northern side of the basin, specifically on the coasts of South Portugal, South and East Spain, South-Eastern France, and West Italy. However, in the southern part of the basin, it is primarily found in Morocco, as well as in North Algeria and North Tunisia [9-12].

According to Fenanne *et al.* *Chamaerops humilis* L. is one of three palm species that grow naturally in Morocco [3]. On average, this dioecious dwarf palm is often acaulescent in its natural habitat, seldom exceeding two meters. However, in cultivation and protected areas, it can reach a height of 10 m [5]; its leaves, petiolate, green, shiny or silvery-glaucous on the upper surface, almost whitish beneath; arranged in a terminal rosette, are fan-shaped, 50-90 cm in diameter; The upper third or half of the leaf blade is deeply divided into 10 to 20 elongated and acute pseudo-leaflets, generally rigid, sometimes a little drooping. Petiole 10 to 50 cm long, with generally spiny margins. Inflorescences are erect spadices up to 15 to 20 cm long. Fruits are spheroidal, ovate-subglobose to obovate and oblong, reddish-yellow to reddish-brown; pericarp fleshy, internally fibrous, and not very thick. The seed is ovate, subglobose, or ± oblong. Generally, berries ripen between September and October [2].

In traditional medicine, the aerial part of *C. humilis* is used to treat pyelonephritis and prostatitis [13]. The fruits and roots are also employed in treating diabetes and digestive system disorders. The fruits are also used as an antidiarrheal remedy for various digestive issues [10].

Chemical studies of extracts from various parts of *C. humilis* revealed the presence of a wide range of bioactive compounds spread throughout numerous phytochemical groups, including lipids, proteins, and crude fibers [14]. It also contains polyphenolic compounds grouped into three main chemical classes: phenols, flavonoids, and tannins [15]. Additionally, terpenes have been identified in these studies [16]. Thus, previous studies on *C. humilis* oil have shown the presence of oleic acid, linoleic acid, sterols, β -sitosterol, and δ -tocopherol [16]. Furthermore, numerous researchers have described the pharmacological effects of *C. humilis* properties and their active compounds. These effects include analgesic, antioxidant, antiviral,

anti-inflammatory, anti-tyrosinase, antibacterial, antidiabetics, antihyperlipidemic, antilithiatics, and anticancer properties [12, 15, 17-19].

This study aimed to quantify the total phenolic, flavonoid, and condensed tannin content as well as assess the anti-inflammatory and antioxidant properties of the pulp extracts of *C. humilis* collected from two different regions in Morocco, Beni Snassen and Khenifra.

2. Materials and Methods

2.1. Plant material.

Ripe fruits of *C. humilis* were gathered from the Khenifra and Beni Snassen regions during the fieldwork conducted in February 2023. The plant was authenticated by Hamid KHAMAR, Scientific Institute, Department of Botany and Plant Ecology, University Mohammed V in Rabat. The plant material (fruit pulp) was placed in a laboratory oven and heated at a controlled temperature of 40°C for a period of 3 days. Following this, it was finely ground into a powder using a high-quality blender. The plant powder was stored under optimal conditions at room temperature

2.2. Preparation of extracts.

2.2.1. Preparation of organic extracts.

Organic *C. humilis* extracts were obtained using the Soxhlet method. A 50 g quantity of pulp powder was sequentially extracted with hexane, dichloromethane, and methanol utilizing a continuous hot extraction procedure facilitated by a Soxhlet extractor. The crude extract was obtained through the evaporation of the acquired solvent utilizing rotavapor. The crude extracts were stored at +4°C until they were needed for applications.

2.2.2. Preparation of aqueous extracts.

The aqueous extracts of *C. humilis* were obtained by a decoction of the plant material (90 g of plant material in 600 ml of distilled water). Afterward, the solvent was evaporated under reduced pressure to yield the crude extract, which was subsequently stored at +4°C until it was needed for further applications.

2.3. Qualitative analysis.

The plant's phytochemical constituents were identified through a preliminary qualitative phytochemical analysis. The different phytochemicals were tested: tannins, saponins, flavonoids, anthraquinone glucoside, triterpenes, and sterols, using established methods as mentioned in the literature [20, 21].

2.4. Determination of phenolic content.

2.4.1. Determination of total phenolic content.

Using Folin-Ciocalteu as an agent, the total polyphenols were quantified according to the protocol outlined by [22]. By following this method, the extracts were diluted to a concentration of 1 mg/mL using methanol. Next, 0.1 mL and 0.5 mL of the 10% Folin-Ciocalteu agent were added to test tubes. After incubating for one hour at room temperature,

we add 2 mL of 2% sodium carbonate (Na₂CO₃). Subsequently, the mixture was incubated once again at room temperature for 30 minutes. Using a similar technique, we established a reference range of 0 to 0.1 mg/mL, starting with an aqueous stock solution of gallic acid (0.5 g/L). An absorbance measurement at 760 nm was obtained using a UV-visible spectrophotometer. The calibration bend for gallic acid was made easier to create because of the absorbance values for each concentration. The results are expressed in milligrams of Gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g). Each manipulation is performed three times.

2.4.2. Determination of total flavonoid content.

The assessment of flavonoids was carried out using aluminum trichloride (AlCl₃) as an agent, following the method of Brighente *et al.* [23]. In this procedure, 1mL of *C. humilis* extract is mixed with 1 mL of the reagent with a concentration equal to 2% and 50 µL of acetic acid. After gently stirring the mixture, the tubes are incubated at room temperature for 40 minutes in the dark. A quercetin stock solution was prepared in methanol at a concentration of 0.2 g/L under comparable conditions. A range of concentrations (0 to 25 mg/ml) were prepared from this solution, and their absorbances were measured at 415 nm. The results allow us to establish the standard curve for quercetin. The results are expressed as milligrams of quercetin equivalent (mg QE) per gram of dry extract (mg QE/g). Every operation was carried out in three trials.

2.4.3. Determination of condensed tannins.

The condensed tannins were assessed using the procedure outlined by Broadhurst and Jones [24] with slight modifications. 500 µL of our extracts, 3 mL of 4% vanillin, and 1.5 mL of concentrated chloric acid were sequentially added in test tubes. The combination was left to settle for 15 minutes at ambient temperature, and the absorption was determined at 500 nm. The content of condensed tannins was expressed in terms of catechin equivalents (mg CE/g of extract). Under the same conditions, various concentrations ranging from 0 to 1000 µg/mL, prepared from a stock solution of catechin, were used to construct the calibration curve. All procedures were conducted in triplicate.

2.5. Antioxidant activities.

2.5.1. DPPH assay.

The antioxidant activity using an in vitro DPPH assay was carried out following the procedure of Kubola and Siriamornpun [25] with some adjustments. Briefly, 0.9 mL of DPPH solution prepared in methanol was introduced into tubes containing 0.1 mL extracts at different concentrations from 0.3125 mg/mL to 5 mg/mL for *C. humilis* extracts. Next, vortex the mixtures and store them at room temperature in the darkness. Then, the absorbance is measured at 517 nm after 30 minutes. Positive controls were conducted using ascorbic acid. The ability to scavenge the DPPH radical was determined by using the following equation (1):

$$A\% = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100 \quad (1)$$

Abs control: Absorbance without antioxidant (containing all reagents except the test sample); Abs sample: absorbance with the test sample.

IC₅₀ value represents the sample concentration required to reduce DPPH radicals by 50%. The IC₅₀ value was obtained using linear regression plots, which included calculating the percentage inhibition against various concentrations of the tested fractions and reference.

2.5.2. ABTS assay.

The antioxidant activity was assessed using the ABTS^{•+} radical, as outlined by Re *et al.* [26], generated by combining 2.4 mM potassium persulphates with 7mM ABTS solution 2.4mM potassium persulfate and left for 16h in the dark, at room temperature. After this time, the ABTS^{•+} solution was reduced in concentration to achieve an absorbance of 0.70 ± 0.02 nm at 734 nm using methanol. The spectrophotometer was initially calibrated with methanol. Next, 1 mL of the diluted ABTS^{•+} solution was added to 0.5 mL of *C. humilis* extracts at various concentrations (ranging from 0.039 mg/mL to 5 mg/ml). The mixture was stored away from light at room temperature. After 7 min, the absorbance was measured at 734 nm. BHT was employed as a positive control.

The ability to scavenge the ABTS^{•+} radical was calculated using the following equation (2):

$$I\% = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100 \quad (2)$$

Where:

Abs control: Absorbance without antioxidant (containing all reagents except the test sample); Abs sample: absorbance with the test sample.

The results were expressed as IC₅₀. A lower IC₅₀ value indicates a more potent antioxidant activity.

2.6. Anti-inflammatory activity.

The in vitro anti-inflammatory activity of the plant extracts was examined utilizing the Bovine Serum Albumin Protein Denaturation Assay (BSA) following the method determined by Kandikattu *et al.* in 2013 [27] with slight adjustments. We added 0.5 ml of BSA solution (0.2%) prepared in Tris Buffered (pH 6.8) for every extract concentration. The mixture was put in the oven at 37°C for 15 min followed by immersion in a water bath at 72°C for 5 min. Once the tubes were cooled, the turbidity (level of protein precipitation) was measured at 660 nm using a spectrophotometer. Diclofenac served as the standard for comparison, and the percentage inhibition of denaturation of the proteins was calculated using the equation:

$$I\% = (\text{Control} - (\text{sample} - \text{White})) / \text{control} * 100$$

Where:

- Sample: 0.5 ml extract + 0.5 ml BSA
- White: 0.5 ml extract + 0.5 ml Tris-phosphate (pH: 6.8)

2.7. Tocopherol content and composition.

The analysis of tocopherol content and composition was conducted utilizing High-Performance Liquid Chromatography (HPLC) in accordance with the **ISO 9936: (2016)** [28] protocol. The HPLC analysis was performed using a Shimadzu SPD-M20A (Shimadzu Corporation, KYOTO, JAPAN) system equipped with a fluorescence spectrophotometer detector and a LabSolutions integration system. A solution containing 250 mg of hexane extract in 25 mL of n-heptane was filtered and directly injected using an auto-sampler onto a Diol phase HPLC column (25 cm × 4.6 mm i.d.) (Merck, Germany) with a flow rate of 1.3 mL/min.

The mobile phase utilized was a 99:1 isooctane:isopropanol (V/V). The excitation and emission detector wavelengths were set at 295 nm and 330 nm, respectively. Identification was based on retention time, and tocopherols were quantified using external standards (α -tocopherol). The results were expressed as milligrams of tocopherols per kilogram of oil.

3. Results and Discussion

3.1. Yields.

The quantity of extractable compounds was proportional to the polarity of the solvent, following the order: water, methanol, and hexane. According to Table 1, it is evident that the extraction yields of *C. humilis* pulps from Beni Snassen are significantly higher compared to those from Khenifra.

A study presented by Mhanni *et al.* showed that the yield of pericarps, grains, and a mixture of pericarps and grains of *C. humilis* oils extracted by the Soxhlet method are, respectively 5.99%, 9.18%, and 6.75% [29].

Table 1. Yields of *C. humilis* extracts.

Extractable compounds	Yields %	
	<i>C. humilis</i> pulps from Khenifra	<i>C. humilis</i> pulps from Beni Snassen
Hexane extract	2.37	3.6
Dichloromethane extract	1.69	1.40
Methanol extract	5.61	7.42
Aqueous extract	48.35	72

3.2. Qualitative analysis.

The phytochemical screening of *C. humilis* pulps from the two regions (Beni Snassen and Khenifra) exposed the existence of tannins, sterols, flavonoids, and triterpenes, as well as the absence of saponins, and anthraquinone glucoside (Table 2). The results of our study exhibited a slight similarity with a study conducted by Benmehdi and co-workers [5]. Those authors revealed the presence of saponins in addition to the alkaloids, flavonoids, terpenoids, steroids, tannins, anthraquinones, reducing sugars, coumarins, fatty acids, and volatiles. Furthermore, a study investigated the phytochemical composition of the powder, and organic and aqueous extracts of *C. humilis* leaves extracts from the Taza region (Morocco) and revealed the presence of the following chemical families: catechins, gallotanins, flavonoids, saponins, alkaloids, sterols, anthracenones, free quinones, and anthraquinones [10]. Another study evaluated the phytochemical screening of the methanolic extracts of the leaflets, rachis, and roots of *C. humilis* and demonstrated the presence of flavonoids, tannins, saponins quinons, and coumarins in all the parts except the roots, which contain few flavonoids [30].

Table 2. Preliminary qualitative phytochemical analysis of *C. humilis* pulps.

	<i>C. humilis</i> pulps from Khenifra	<i>C. humilis</i> pulps from Beni Snassen
Flavonoids	+	+
Tanins	+	+
Saponins	-	-
Sterols and triterpens	+	+
Anthraquinone glucoside	-	-

+: Presence; -: Absence.

3.3. Phenolic content.

3.3.1. Total phenolic content.

The contents of total polyphenols were assessed through the colorimetric method utilizing the Folin-Ciocalteu agent. The findings are presented in mg GAE/g extract utilizing the calibration curve established earlier with gallic acid (polyphenol correlation: $R^2 = 0.998$) as a reference. Tables 3 and 4 outline the polyphenol content results for extracts of *C. humilis* pulps.

The aqueous extract exhibited the highest total polyphenol content for Khenifra and Beni Snassen pulps, with values of 41.808 ± 0.02 mg GAE/g and 48.676 ± 0.013 mg GAE/g, respectively. On the other hand, the methanol, dichloromethane, and hexane extracts showed the lowest polyphenol content.

The concentration of polyphenols in other studies was lower compared to our extract. The study presented by Eddahhaoui *et al.* [15] reports that the methanol fraction of *C. humilis* from the pulps contains 35.81 ± 0.42 mg GAE/g of polyphenols. Recently, a study conducted by Nakhela *et al.* (2023) evaluated the polyphenols content in *C. humilis* pulps extract and showed a value of 15.848 ± 0.27 mg GAE/g dry weight [31]. In addition, other studies reported the total polyphenol content of other parts of *C. humilis*. According to the study conducted by Bouhafoun *et al.*, the total polyphenol of leaflets, rachis, and roots contents as 26.8 ± 0.41 mg GAE/g, 28.7 ± 0.44 mg GAE/g, and 26 ± 0.50 mg GAE/g of polyphenols, respectively [30]. Recently, Mhanni *et al.* evaluated the total polyphenolic content in seed extract and showed a value of 34.224 mg GAE/ g DM [29].

3.3.2. Total flavonoid content.

The trichloride aluminum agent assesses total flavonoids, and the findings are reported in mg QE/g extract derived from the established calibration curve with quercetin as a reference (flavonoid correlation: $R^2 = 0.999$). The flavonoid contents of *C. humilis* extracts are outlined in Table 3 and Table 4.

The flavonoid contents varied among the two regions, ranging from 4.456 ± 0.002 to 0.635 ± 0.005 mg QE/g of extract (Table 3, and Table 4). The hexane extract presented the highest flavonoid content of 4.456 ± 0.002 mg QE/g for the Beni Snassen region and 2.367 ± 0.001 mg QE/g for the Khenifra region. Alternatively, the methanol extract had the lowest flavonoid content value. Several studies have reported the flavonoid content of *C. humilis* extract. A study conducted by Gonçalves *et al.* [32] reported that the flavonoid content of methanol extracts from *C. humilis* pulp was 19.48 ± 0.64 $\mu\text{mol/g}$ of extract, a value lower than that obtained in our study. In 2021, El Cadi *et al.* evaluated the flavonoid content in *C. humilis* fruits collected from northeast Morocco. The results showed that the flavonoid content was 11.1 ± 0.45 mg EQ/g for the methanol-water extract and 6.5 ± 0.1 mg EQ/g for the ethyl acetate extract [33]. Recently, a study conducted by Nekhla *et al.* evaluated the total flavonoid content in *C. humilis* pulps extract and showed a value of 0.208 ± 0.01 mg GAE/g dry weight [31]. The extraction method used in the different studies could explain these differences in values. Similarly, several factors can influence the flavonoid content, including external factors (e.g., climatic and geographical factors) and the degree of plant maturity [34, 35].

3.3.3. Condensed tannins content.

The condensed tannins are determined by vanillin and chloric acid, and the results are presented in mg CE/g extract derived from the developed calibration curve with catechins (correlation: $R^2 = 0.999$). The findings on the condensed tannin contents of *C. humilis* pulps extracts are outlined in Tables 3 and 4.

Table 3. Total phenolic, flavonoids, and condensed tannins content of pulps extracts of *C. humilis* from Khenifra (Morocco).

	<i>C. humilis</i> pulps from Khenifra		
	Polyphenols (mg of GAE/g of extract)	Flavonoids (mg of QE/g of extract)	Condensed tannins (mg of CE/g of extract)
Hexane extract	18.171 ± 0.015	2.367 ± 0.001	0.164 ± 0.013
Dichloromethane extract	22.818 ± 0.005	1.262 ± 0.001	0.138 ± 0.222
Methanol extract	24.434 ± 0.004	0.755 ± 0.006	0.0103 ± 0.003
Aqueous extract	41.808 ± 0.024	1.083 ± 0.002	0.044 ± 0.005

Table 4. Total phenolic, flavonoids, and condensed tannins content of pulps extracts of *C. humilis* from Beni Snassen (Morocco).

	<i>C. humilis</i> pulps from Beni Snassen		
	Polyphenols (mg of GAE/g of extract)	Flavonoids (mg of QE/g of extract)	Condensed tannins (mg of CE/g of extract)
Hexane extract	18.979 ± 0.007	4.456 ± 0.002	0.174 ± 0.047
Dichloromethane extract	27.262 ± 0.014	1.217 ± 0.005	0.075 ± 0.013
Methanol extract	32.212 ± 0.0126	0.635 ± 0.005	0.024 ± 0.005
Aqueous extract	48.676 ± 0.013	1.068 ± 0.004	0.073 ± 0.018

The results show that the highest condensed tannin contents are observed in hexane extracts (0.174 ± 0.047 mg CE/g of extract, 0.164 ± 0.013 mg CE/g of extract for the Beni Snassen and Khenifra regions, respectively), followed by dichloromethane extracts (0.075 ± 0.013 mg CE /g of extract, 0.138 ± 0.22 mg CE /g of extract for the fruit pulps from Beni Snassen and Khenifra, respectively). Conversely, methanol extracts present the lowest content. Several researchers have reported the condensed tannin contents of *C. humilis* extracts. According to Gonçalves *et al.* [32], the condensed tannins content in the methanol extract of *C. humilis* pulps, leaves, and peel collected from the south of Portugal were 61.27 ± 5.12 $\mu\text{mol/g}$ of extract, 38.75 ± 6.06 $\mu\text{mol/g}$ of extract, and 111.46 ± 9.78 $\mu\text{mol/g}$ of extract, respectively. The variation in proanthocyanidin content can be explained by the influence of several parameters, including extrinsic factors.

3.4. Antioxidant activities.

3.4.1. DPPH assay.

The results of the DPPH test for the extracts of the two studied regions of *C. humilis* show that the inhibition percentage is proportional to the concentration of the extracts (Figures 1 and 2). Antioxidant activity was evaluated using an *in vitro* DPPH assay. The findings are presented in ascorbic acid equivalents. The DPPH tests of various extracts of *C. humilis* from two studied regions at different concentrations are presented in Figures 1 and 2. All extracts demonstrated a scavenging activity that rises with the concentration. A reduced value of IC_{50} suggests a high antioxidant activity. Notably, the aqueous *C. humilis* extracts from the Beni Snassen and Khenifra regions (Morocco) registered high antioxidant activity ($\text{IC}_{50} = 0.542 \pm 0.141$ mg/mL and 1.381 ± 0.1448 mg/mL, respectively) followed by the methanol extracts.

Dichloromethane and hexane extract of *C. humilis* have an $IC_{50} > 5$ mg/mL (Table 5). The IC_{50} value of ascorbic acid was $BHT \pm 0.17$ μ g/mL. The high antioxidant activity of ascorbic acid can be attributed to its use as a pure molecule compared to our extract.

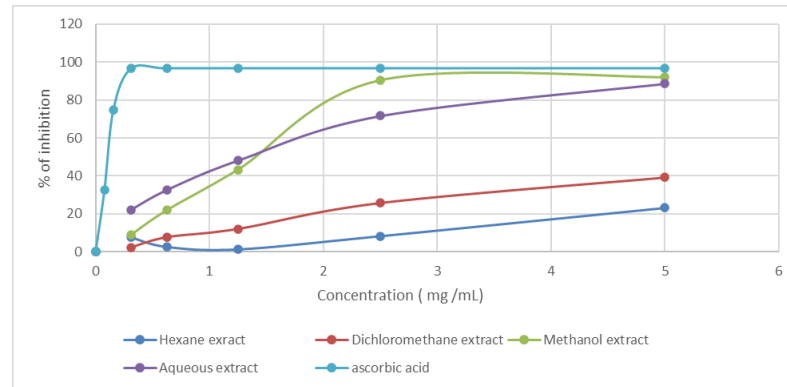


Figure 1. DPPH radical scavenging activity of *C. humilis* extracts (Khenifra, Morocco).

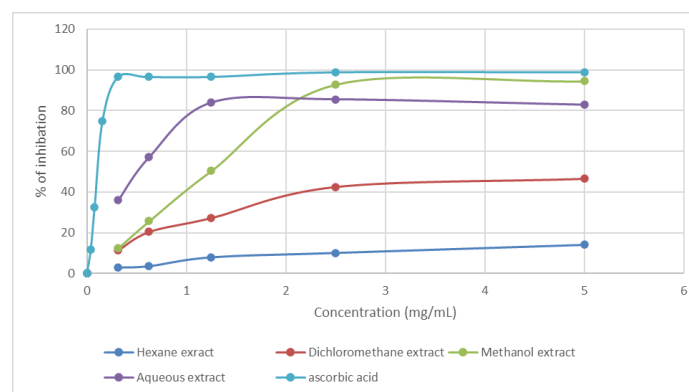


Figure 2. DPPH radical scavenging activity of *C. humilis* extracts (Beni Snassen, Morocco).

Table 5. Free radical (DPPH) scavenging of *C. humilis* extracts.

Extracts	IC_{50} (mg/ml)	
	<i>C. humilis</i> pulp collected from Khenifra	<i>C. humilis</i> pulp collected from Beni Snassen
Hexane extract	$IC_{50} > 5$	$IC_{50} > 5$
Dichloromethane extract	$IC_{50} > 5$	$IC_{50} > 5$
Methanol extract	1.776 ± 0.462	1.27 ± 0.021
Aqueous extract	1.381 ± 0.144	0.542 ± 0.141

Several studies have reported the capacity of organic extracts of diverse parts of *C. humilis* to scavenge free radicals using the DPPH test. An analysis conducted by Gonçalves *et al.* in 2018 reported that the IC_{50} value in the methanol extract of *C. humilis* pulps collected in Spain and extracted using the maceration method was 325.03 ± 10.81 μ g/ml, which is a lower concentration than that obtained in our study [32]. In 2013, another study by Miguel *et al.* on the antioxidant activity of *C. humilis* leaves exhibited the strongest antioxidant activity ($IC_{50} = 0.035$ mg/ml) [36]. Recently, a study conducted by Nekhela *et al.* studied the antioxidant effect (DPPH assay) of pulps extract using the sonication method and showed an $IC_{50} = 5.526 \pm 0.29$ mg/ml [31]. This difference in IC_{50} values can be attributed to the extraction method used and the plant's geographical region [37, 38].

3.4.2. ABTS assay.

The results of the ABTS test for the two studied regions of *C. humilis* show that the inhibition percentage is proportional to the concentration of the extracts (Figures 3, 4, 5, and 6).

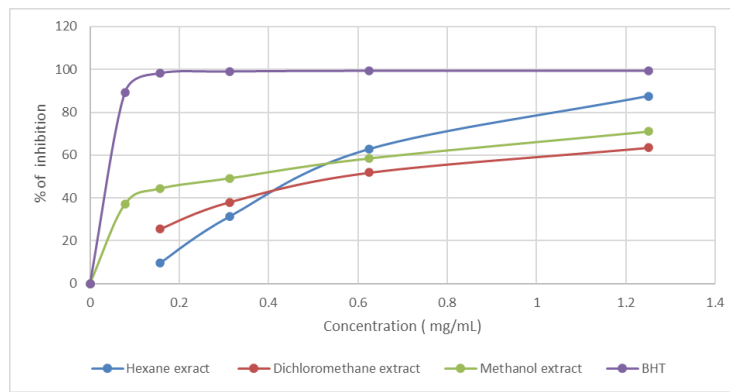


Figure 3. ABTS⁺ radical scavenging activity of *C. humilis* organic extracts (Khenifra, Morocco).

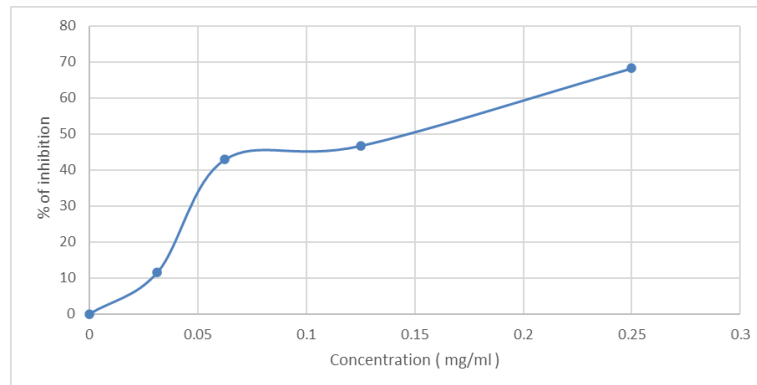


Figure 4. ABTS⁺ radical scavenging activity of *C. humilis* aqueous extracts (Khenifra, Morocco).

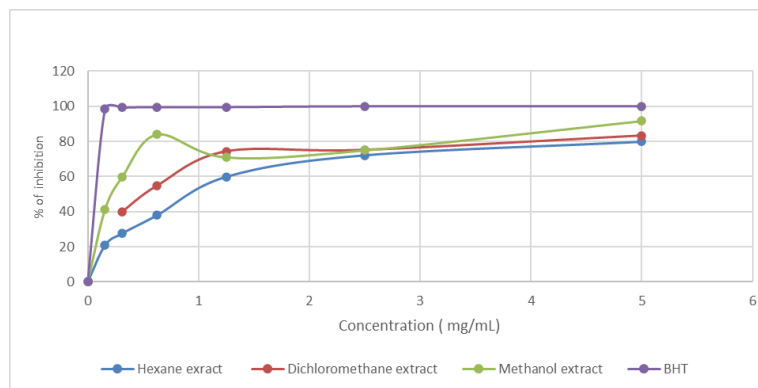


Figure 5. ABTS⁺ radical scavenging activity of *C. humilis* organic extracts (Beni Snassen, Morocco).

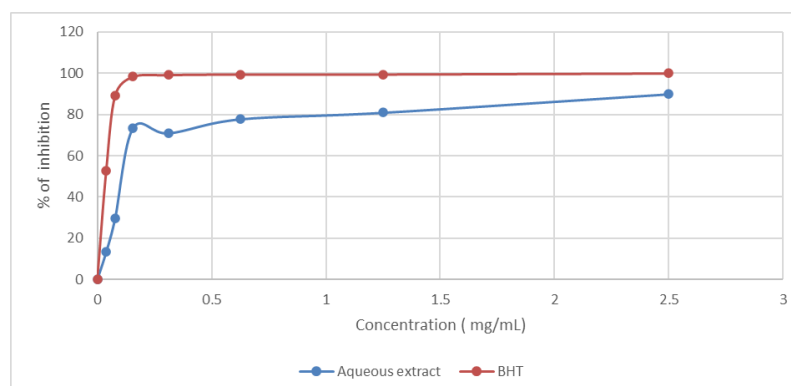


Figure 6. ABTS⁺ radical scavenging activity of *C. humilis* aqueous extracts (Beni Snassen, Morocco).

The assessment of antioxidant activity was conducted using *in vitro* ABTS techniques. The results demonstrate that the aqueous extracts of the *C. humilis* pulp from Beni Snassen and Khenifra (Morocco) showed the strongest ability to scavenge ABTS⁺ radicals (Figures 4 and

6), with IC₅₀ values of 0.11 ± 0.005 mg/ml, and 0.19 ± 0.08 mg/ml, respectively, followed by the methanol fractions than the hexane and dichloromethane extracts (Table 6). Furthermore, it is necessary to note that the pulps of *C. humilis* from Beni-Snassen exhibit a slightly higher percentage than that from Khenifra. His use can interpret the high antioxidant activity of BHT as a pure molecule compared to our extract, which contained several molecules. However, the antioxidant activity of *C. humilis* utilizing the ABTS assay has been used before. [36] reported IC₅₀ values of 0.035 ± 0.079 mg/mL using the ABTS assay, with different extracts prepared from aerial parts of *C. humilis* collected in Morocco. A study conducted by Gonglaves [32] showed that the IC₅₀ value of the methanol extract of *C. humilis* pulps was 351.06 ± 11.99 µg/ml, which is a lower concentration than our present study's findings.

Table 6. Free radical (ABTS⁺) scavenging of *C. humilis* extract.

Extracts	IC ₅₀ (mg/ml)	
	<i>C. humilis</i> pulp collected from Khenifra	<i>C. humilis</i> pulp collected from Beni Snassen
Hexane extract	0.53±0.080	0.83±0.18
Dichloromethane extract	0.60±0.13	0.53±0.109
Methanol extract	0.32±0.08	0.24±0.11
Aqueous extract	0.19±0.08	0.11±0.005

3.5. Anti-inflammatory activity.

The study of the anti-inflammatory activity of our extracts was performed *in vitro* by evaluating the inhibition of BSA denaturation. This study represents the first investigation into the anti-inflammatory activity of the aqueous extract of *C. humilis* pulps using BSA protein. According to the results obtained from the aqueous extracts of *C. humilis* pulps from both regions (Figure 7), a direct correlation was observed between the extract concentration and the inhibition of BSA denaturation. Indeed, an inhibition of 50% was observed in a concentration range from 0.3 mg/ml to 0.61 mg/ml. Furthermore, the pulps from the Beni-Snassen region showed slightly higher inhibition compared to the pulps from Khenifra. Despite the high anti-inflammatory activity, the observed effects are significantly lower than the inhibitory effect demonstrated by the standard molecule diclofenac. For this molecule, a 50% inhibition is achieved at a specific concentration of 0.098 ± 0.001 mg/ml (Figure 8). This can be explained by the use of a pure molecule in the case of diclofenac. At the same time, our aqueous extracts contain multiple molecules, and the concentration of the one responsible for the anti-inflammatory effect is likely too low compared to the actual concentration of diclofenac.

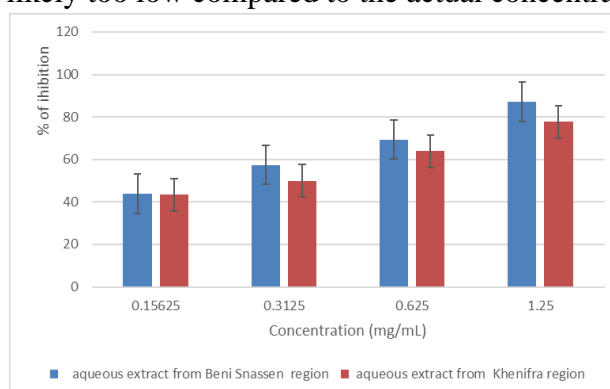


Figure 7. Percentage of inhibition of BSA denaturation by the aqueous extracts of both regions of *C. humilis*.

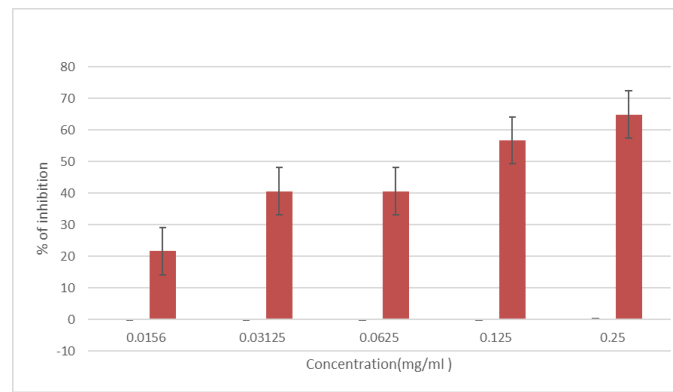


Figure 8. Percentage of inhibition of BSA denaturation by Diclofenac standards.

A scientific study was conducted by Miguel *et al.* using *in vitro* 5-lipoxygenase assay. Investigated the anti-inflammatory activity of *C. humilis* and showed an IC₅₀ value of 16 ± 0.030 mg/ml [36]. The variation in IC₅₀ values can be attributed to the utilization of diverse assays and the geographical region from which the plants were sourced. Recently, a study reported the anti-inflammatory activity of aqueous fruits *C. humilis* extracts using human serum albumin (HAS) and showed an IC₅₀ =446.02 mg/ml [39].

3.6. Tocopherol composition.

Tocopherols are essential to analyze because of their vitamin, nutritional, and free radical preservation properties. These natural lipophilic antioxidants exist in the plant lipid fraction in four forms: α-tocopherol (vitamin E), β-tocopherol, δ-tocopherol, and γ-tocopherol. Their role is crucial because they can intercept free radicals, making them vital in nutrition. Table 7 below displays the quantification of tocopherol in *C. humilis* pulps, as determined by this research. The lipophilic fraction was found to contain a total tocopherol of 3118.51 ± 3.99 mg/kg in samples collected from Khenifra, while samples collected from Beni-Snassen had a level of 3408.98 ± 3.76 mg/kg. These values were higher than those reported by Mokbili *et al.* and Arbi Nehdi *et al.* from Tunisian *C. humilis* (880 mg/kg and 750 mg/kg, respectively) [40, 41].

Table 7. Tocopherol composition and content of *C. humilis* pulps hexane extract.

	Tocopherol contents (mg/kg)	
	Hexane extract of <i>C. humilis</i> (from Khenifra)	Hexane extract of <i>C. humilis</i> (from Beni-Snassen)
α-tocopherol	2333.05±3.71	2402.35±2.23
β-tocopherol	136.342±1.82	190.78±1.84
γ-tocopherol	446.557±1.46	638.67±2.01
δ-tocopherol	202.556±1.07	177.18±1.15
Total tocopherols (mg/kg)	3118.51±3.99	3408.98±3.76

Four tocopherols were identified in *C. humilis* pulps, with α-tocopherol as the main compound (CHK: 2333.05 ± 3.71 mg/kg and CHBS: 2402.35 ± 2.23 mg/kg) followed by γ-tocopherol (CHK: 446.557 ± 1.46 mg/kg and CHBS: 638.67 ± 2.01 mg/kg), δ-tocopherol (CHK: 202.556 ± 1.07 mg/kg and CHBS: 190.78 ± 1.84 mg/kg), and β-tocopherol (CHK: 136.342 ± 1.82 mg/kg and CHBS: 177.18 ± 1.15 mg/kg) identified as minor tocopherols for both samples A slight variation was observed between the two samples studied. According to Nounah *et al.*, this difference may be attributed to the geographical origin of the plant material [42]. Interestingly, compared to other studies [40-42], our analysis revealed a significant difference in terms of individual tocopherol. This indicated the presence of α-tocotrienol as the major compound; however, it was not detected in our samples.

4. Conclusion

The phytochemical analysis performed on *C. humilis* from the Beni Snassen and Khenifra in Morocco revealed the presence of flavonoids, tannins, sterols, triterpenes, but the absence of saponins and anthraquinone glucoside. The reported results show that the aqueous extract of *C. humilis* pulps from the two regions contains the highest concentration of polyphenol compounds and exhibits great antioxidant activity through scavenging ability according to DPPH and ABTS methods. In addition, the assessment of the anti-inflammatory activity involved measuring the inhibition of bovine serum albumin (BSA) denaturation due to heat. The aqueous extracts of both regions showed a significant capacity for inhibiting BSA denaturation, while the second variety exhibited a higher inhibitory power compared to the first one. The various results of this study indicate that the aqueous extracts of *C. humilis* pulp from both regions showed anti-inflammatory and antioxidant activities. Moreover, *C. humilis* from the Beni Snassen region exhibits a higher antioxidant and anti-inflammatory potency compared to *C. humilis* from the Khenifra region, possibly attributed to its geographical origin.

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Conflicts of Interest

The authors declare no conflict of interest.

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