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BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS OF *CHAMAEROPS HUMILIS* L.

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Abstract

Medicinal plants are an important source of bioactive molecules that are known for their therapeutic properties. *Chamaerops humilis* L. *(C. humilis)* among the most traditionally used therapeutic plants in Morocco, often found in the Mediterranean areas. Nowadays, medicinal plants are gradually helping to replace synthetic drugs, in order to weaken the aggressive effects of said compounds on the human body. In this study, *C. humilis* was collected in the zaer region, the extracts of the selected parts are prepared by soxhlet extraction method using methanol as solvent. Phytochemical analysis revealed the existence of tannins, flavonoids, saponins, terpenoids, and coumarins. The quantification of phenolic compound showed that the roots extract is the richest (151.09±2.83 mg GAE g^{-1} of extract), while palm heart extract is the poorest (79.12±2.17 mg GAE g^{-1} of extract). Evaluation of the antioxidant activities (AA) using the DPPH, ABTS and FRAP tests indicated that methanolic root extract has good antioxidant efficacy with IC_{50} values of 1.99±0.02 µg mL^{-1}, 37.65±0.66 µg mL^{-1}, and 279.61±4.90 µg mL^{-1}, respectively. The results of the various tests carried out are promising for a possible valorization of *C. humilis* as a bioresource in the therapeutic field.

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Introduction

For a long time, man has relied on the resources of nature, especially plants, for his food and medicine needs. Today, much research is being conducted on natural substances and their potential medicinal use (RHATTAS 2016 et al. 2016). It is estimated that more than 70% of medicines are derived from plant substances. Nearly 170,000 organic molecules have been identified from plants: quinine, digitaline, colchicine etc, less than half of these compounds are intended for therapeutic uses (CHAABI 2008). These organic molecules are being extensively studied for their potential use as remedies for chronic diseases such as cancer, kidney stones, ulcers and diabetes (PASSALACQUA 2006, RAMMALl 2009, SQALLI 2007). *C. humilis*, is a concrete example of a valuable natural resource because it has high ethno-pharmaceutical value (SQALLI and POLLIO 1994, BEGHALIA 2008, BENMEHDI 2012, HASNAOUI 2011). Doum is the Moroccan Arabic name of our studied plant. It belongs to the tribe of *Livistoneaes*, sub-tribe of *Raphidinaes*, of the genus *Chamaerops*, species *Chamaerops humilis* (DRANSFIELD et al. 2005). It is a floristic plant widespread in many countries of the western Mediterranean (REENÉ 1957). *C. humilis* is a well-known species since it is the only species of its family in the northern Mediterranean (QUÉZEL et al. 1999). It avoids areas that are too wet or too dry and grows spontaneously. *C. humilis* has several medicinal and non-medicinal uses. It is an ornamental plant, a source of fiber for domestic and artisanal uses (MOTTI 2009, SAVO 2013). It is also a dietary source whose heart, young shoots and fruit are edible and rich in nutrients (TARDÍO et al. 2006). Aqueous *C. humilis* leaf extract is traditionally used to treat diabetes, as it is thought to reduce total cholesterol and triglyceride levels. The traditional use of this plant suggests that it may be a potential source of medication and may be useful in the management of secondary complications of diabetes and gastrointestinal disease (GAAMOUSSI 2010). Furthermore, the fruits of *C. humilis* are used as antiseptics, while the roots are used to treat anemia and intestinal worms. These uses can be supported by the fact that the alcoholic extract of this plant has been shown to be rich in phenolic compounds that have an interesting antioxidant action (KHOUDALI 2014).

Our work is based on a comparative study between five different parts of the dwarf palm, four underground parts include the fibers, bark, roots and heart of the palm, and the other aerial part includes the leaves.

Materials and Methods

Preparation of extracts

The studied parts of C. *humilis* are collected in the Zaer region (75.9 km to Rabat, Morocco) in December 2018, the different parts dried for about ten days in the shade. 15 g of mixed material from each sample are placed in a cellulose cartridge and subjected to extraction by the soxhlet for 6 hours with hexane, followed by a second extraction with methanol for 4 hours. The extracts are then concentrated under vacuum using a rotary vacuum evaporator (Heidolph G1, Germany) at 60–75°C and the dried residues are stored at -4°C for further studies.

Moisture determination

Moisture is the amount of water contained in plant matter. This parameter was measured by the oven drying method at 105±5°C. To ensure good preservation, the moisture content must be less than or equal to 10% (SHARIFIFAR et al. 2007). The percentage of moisture is calculated by the following formula:

$$M\,[\%] = \frac{(m_1 - m_2)}{m_1} \cdot 100$$

where:
M – percentage of moisture
m_1 – weight of the sample in grams after harvesting of the fresh plant.
m_2 – weight of sample in grams after drying (dried plant).

Phytochemical screening

Phytochemical screening is a qualitative test based on precipitation and turbidity reactions or color change. These reactions are used to determine the presence or absence of secondary metabolites in the *C. humilis* parts.

We characterized the different chemical groups using methanolic extracts following the techniques as described in published literature (CHATOUI 2016, LABIAD 2017).

Alkaloids

Alkaloids are detected in extracts with the Mayer reagent by a precipitation reaction. Each methanolic extract was dissolved in a few mL of 50% hydrochloric acid, the formation of a yellow precipitate after the addition of drops of reagent indicates the presence of alkaloid (ABBAS 2014).

Tannins

1.5 g of each dry extract is mixed with 10 mL of methanol (80%), and stirred for 15 minutes; the extracts are filtered and placed in test tubes. The presence or absence of tannins is detected by the addition of 1% $FeCl_3$. The blue-black and green-brown color indicates the presence of gallic and catechic tannins respectively (DOHOU et al. 2003).

Flavonoids

The detection of flavonoids is based on the reaction of cyanidin. Two mL of extract are vaporized, and the residue is absorbed in 5 mL of HCl-EtOH (2:1, v/v). 2 to 3 parts of magnesium are added to cause heat release, which subsequently results in pink or purplish coloration. This coloration intensifies after the addition of 3 drops of isoamyl alcohol, which indicates a positive reaction (AZZI 2013).

According to Liebermann's reaction, 5 mL of each extract is vaporized on a sand bath. The residue is dissolved hot in 1 mL of acetic anhydride. The addition of 0.5 mL of concentrated sulphuric acid produces a purple color which changes to blue and then green indicating the existence of polyterpenes and sterols (YAM et al. 2009).

Coumarins

In a test tube, 0.5 mL of sodium hydroxide solution (10%) is added to 2 mL of the extract dissolved in methanol, and heated to boiling. Then 4 mL of pure water are added to the mixture. When the tube becomes transparent, this reflects the presence of coumarins.

Saponins

The mixture of 1 mL of the extract with two mL of hot pure water was stirred vigorously for 15 seconds and then left to stand for a quarter-hour. Stable and persistent foam greater than one centimeter in height indicates the presence of saponins (BEKRO 2007).

Starch

10 mL of a saturated solution of NaCl are added to the aqueous extract (1 mL). After heating, starch is added as a reagent, if the mixture turns a purplish blue, it indicates the presence of starch.

Protein

Proteins are detected in the residues of certain parts of *C. humilis* by the biuret reaction. A few milligrams of each residue are dissolved in two mL of the aqueous solution of NaOH 20%, then 2 to 3 drops of a 20% aqueous solution of $CuSO_4$. The appearance of a purple color, sometimes with a reddish tinge, indicates a positive reaction.

Determination of secondary metabolites

Total phenolic content (TPC)

The determination of polyphenols content of methanolic extracts was carried by UV-Visible spectrophotometry using the Folin Ciocalteu reagent (F-C) method (LISTER 2001). In a test tubes, 0.5 mL of sample solution was mixed with 0.5 mL of the aqueous solution of Folin (10%) and 4 mL of Na_2CO_3 (7.5%). The tubes were placed for 30 minutes in a water bath at 45°C. The absorbance was measured at 765 nm. TPC was estimated using a standard gallic acid reagent curve; results were expressed as milligram gallic acid equivalent per gram of extract (mg GAE g^{-1} E).

Total flavonoids content (TFC)

The aluminum trichloride ($AlCl_3$) method is used to determine the flavonoids content (RIAZ et al. 2009). The test consisted in adding 1 mL of sample solution prepared in methanol with 6.4 mL of pure water and 0.3 mL of $NaNO_2$ 5%, after 5 minutes, 0.3 mL of $AlCl_3$ solution at 10% was added, the whole was left for 6 minutes before adding 2 mL of NaOH solution (1 M). The absorbance was measured at 510 nm after 30 minutes of incubation. The TFC was calculated using a standard quercetin curve (mg QE g^{-1} E).

Total tanins content (TTC)

The vanillin acid method consists of determining the condensed tannin content of each extract (SUN 1998). The assay consists of mixing 100 µL of the methanolic solution of the sample with 3 mL of the vanillin solution prepared in methanol (4%), 1.5 mL of hydrochloric acid (HCl) (37%) is added to the mixture, the latter is put 20 minutes in the dark, the absorbance was read at 500 nm by spectrophotometer. The results were expressed as milligram of catechin equivalent per gram of extract (mg CE g^{-1} E).

Antioxidant capacity

Radical scavenging activity of 1,1-diphenyl picryl hydrazyl (DPPH)

The DPPH• test measures the antioxidant power of plant extracts using an organic solvent. This test consists in reducing the free radical DPPH• by hydrogen transfer, the reaction involved causes a transformation of the blue color of the DPPH solution to pale yellow. Briefly, 0.5 mL of the 0.2 mM methanolic DPPH solution was added to 2.5 mL of the stock extract solution at different concentrations and standards (Ascorbic Acid and Trolox). The solution of each sample is then incubated for 30 min in the dark, the absorbance was measured at 517 nm compared to blank samples (EL MOUDDEN et al. 2019).

ABTS radical scavenging test

The following method is used to reduce the cationic radical $ABTS^{+\bullet}$. This method is described by ARNAO (2001). The procedure consists in preparing a solution of 2 mM ABTS and 70 mM potassium persulfate ($K_2S_2O_8$) in equal volumes and leave it stirred in the dark for 12 to 16 hours at ambient temperature. The solution obtained should be read at an absorbance of (0.70±0.02) at 734 nm by dilution in methanol. 2 mL of the prepared solution was added to 200 µL of the methanolic solution of the extracts and the standard (Trolox) at different concentrations, the absorbance was known at 734 nm after 30 min. The difference in absorbance between the ABTS solution in the presence and absence of the sample for the observation of the potential of the compounds responsible for this activity to reduce this radical.

Calculation of the antioxidant activity

The antioxidant capacity by two methods DPPH and ABTS was then calculated using the following formula:

$$\text{AA [\%]} = \frac{(\text{Abs } C - \text{Abs } E)}{\text{Abs } C} \cdot 100$$

where:
Abs C – control absorbance
Abs E – absorbance of the extract.

The inhibitory concentration (IC_{50}) was determined as the concentration of the extract which produces 50% of the trapping effect of the free radicals (DPPH and ABTS).

Reducing power activity (FRAP)

The reduction reaction of ferric ions to ferrous ions is a method determined and modified by TOPÇU et al (2007). It consists of mixing 1 mL of stock solution of the studied extract and standard (ascorbic acid) at different concentrations with 2.5 mL of a 0.2 M phosphate buffer solution at pH = 6.6, and 2.5 mL of a potassium ferricyanide solution $K_3Fe(CN)_6$ (1% w/v). The mixture is incubated for 20 minutes in a water bath at 50°C. The reaction is then stopped by adding 2.5 mL of trichloroacetic acid TCA (10% w/v). The tubes are put in the centrifuge for 10 min at 3000 rpm. 2.5 mL of the supernatant is collected and mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% aqueous solution of ferric chloride ($FeCl_3 \cdot 6H_2O$). The reading of the absorbance is done at 700 nm against a blank prepared in the same manner by pure water.

The effective concentration (EC_{50}) was defined at absorbance 0.5 of the graph. This parameter allows to compare and identify the reducing capacity of the bioactive molecules contained in each extract studied.

Data analysis

The analysis of variance ANOVA was realized by the statistical analysis software IBM SPSS Statistics 21, to validate the statistical significance by Tukey's test at 95.0% confidence level, The values are given as of triplets means ± standard error of the mean. The correlation between all the results of the variables of the studied extracts of *C. humilis* was performed by Pearson's correlation. As well as the combination of the variables with the extracts of this study was done by PCA in the form of a graphical representation, and that of HCA was done to pursue the relationships between all the samples in clusters based on the characteristics of the bioactive agents measured. These three multivariate statistical treatments are performed by XLSTAT 2014 software (ZIELINSKI 2014).

Results and Discussion

Moisture content and extracts yields determination

The results of moisture content are presented in Table 1 and indicate a variation from 29.56±1.48 to 68.96±3.31%, in the different part of the *C. humilis*. There is no significant difference in the moisture content of fibres, barks and roots, however palm hart records the high moisture content, while leaves have a moisture content of 50.10±3.33% (Table 1). These values suggest short-term storage.

Table 1

Moisture and Extracts yields of different parts of *C. humilis*

Specification	Moisture content [%]	Extract yield	
		hexanic extract [%]	methanolic extract [%]
Fibers	34.66 ± 1.98^{a}	3.11 ± 0.34^{a}	12.78 ± 1.30^{a}
Barks	31.71 ± 1.34^{a}	2.65 ± 0.32^{ba}	25.24 ± 2.48^{b}
Roots	29.56 ± 1.48^{a}	$2\ .51\pm0.12^{ab}$	15.70 ± 2.21^{ac}
Palm heart	68.96 ± 3.31^{b}	1.12 ± 0.08^{c}	16.78 ± 1.10^{abc}
Leaves	50.11 ± 3.33^{c}	1.63 ± 0.07^{bc}	22.98 ± 1.42^{bc}

The means are presented as triplicate (n = 3e±SEM), values followed by the same letters in the same column are not different ($P < 0.05$)

The extraction method must allow the extraction of a maximum of phenolic compounds without alteration (HAYOUNI 2007). The solubility of phenolic compounds is governed by their nature, by the extraction method chosen and by the polarity of the solvents used. (GOLI 2005, ROBY 2013, SULAIMAN et al. 2011).

Table 1 showed that there was a significant difference between the extraction rate according to the part of the plant and the solvent used. A high yield of extract was found in the solvent with high polarity (methanol). While hexane that extracting lipid fraction was in low yield. The same results were found by GOLI et al. (2005) for pistachio, the extraction rate increases according to the polarity of the solvent. Methanol is a solvent of high polarity, which explains the relatively high total polyphenol contents recorded in methanolic extracts (ROBY et al. 2013).

Phytochemical screening

Test samples were screened phytochemically for coumarins, proteins, alkaloids, tannins, flavonoids, starches, saponins and terpenoids. The results are shown in Table 2.

Table 2

Phytochemical screening of different parts of *C. humilis*

Specification	Fibers	Barks	Roots	Palm heart	Leaves
Coumarins	+	+	+	++	+
Proteins	-	-	-	-	-
Alkaloids	-	-	-	-	-
Catechic tannins	++	+++	+	-	+++
Gallic tannins	+	+	+	-	++
Flavonoids	++	++	+++	+	++
Starchs	-	-	-	-	-
Saponins	++	++	+++	++	++
Sterols and polyterpenes	+	+	+	++	+++

The results are interpreted as follows: (+) weak presence, (++) medium presence, (+++) strong presence and (-) absence

The phytochemical evaluation of the methanolic extracts of the five parts of *C. humilis* shows that it contains: tannins, coumarins, saponisides, flavonoids, and terpenes. The presence of the metabolites present in each part of the plant differs according to the degree of coloration or the quantity of the precipitate.

Phytochemical tests carried out on the methanolic extract of C. *humilis* swarm leaves confirmed the presence of flavonoids, tannins, saponins, terpenoids, and the absence of steroids (BENMESSAOUD et al. 2018). The results obtained are similar to those reported previously in Algeria (Tlemcen region) and Morocco (Benslimane region) (BENMEHDI et al. 2012, KHOUDALI et al. 2014). This means that the origin has no influence on the presence of the chemical families, but the content of each metabolite may vary from one region to another. In addition, extracts of C. *humilis* roots from Algeria indicated the presence of phenolic compounds, flavonoids, quinons, tannins, saponins and coumarins in the leaflet (BENAHMED-BOUHAFSOUN 2013).

Since C. *humilis* contains flavonoid, it is likely to have antitumor, anticarcinogenic, anti-inflammatory, hypotensive and diuretic activities (JEAN 2009). C. *humilis* contains also coumarins, which have different effects on plant development depending on their concentration and also depending on the species. They are considered phytoalexins, the metabolites that the plant synthesizes in large quantities to fight infections caused by fungi or bacteria and they also have antiedematous and vasoprotective activities (HOFFMANN 2003).

It should also be mentioned that the chemical family of tannins, found also in C. *humilis,* has antiviral, antibacterial and anti-tumor activity. It has also been reported that some tannins are used as diuretics (EZEABARA et al. 2014). Saponins, which have been detected in the extract of parts of C. *humilis,* are responsible for many pharmacological properties, such as inhibitory effects on inflammation (ESTRADA 2000, JUST et al. 1998).

Total phenolic, flavonoid and tannin content (TPC, TFC, and TTC)

Phenolic compounds are active principles responsible for antioxidant powers in biological systems, by dint of its redox properties, which can play an important role in the neutralization of free radicals by absorption, the decomposition of peroxides, or the deactivation of singlet and triplet oxygen (ARORA and CHANDRA 2011). The quantitative study of our different extracts aims to determine total phenolic content: TPC, total flavonoid content: TFC and total tannins content: TTC.

The total phenolic content was varied from 79.12±2.17 to 151.09±2.83 mg EAG g^{-1} extract. The root is the richest part of phenolic compounds. No significant difference ($p > 0.05$) between fibers, bark and leaves extracts were observed, while palm heart extracts had lower values (79.12±2.17 mg GAE g^{-1} E). The results obtained were lower than those reported by BENMESSAOUD et al. (2018), for the leaves (125.847 mg GAE g^{-1} E). While the results of BENAHMED-BOUHAFSOUN et al. (2013) showed lower total content in roots and leaves with values of 26 to 28.7 mg GAE.g^{-1} of extract, respectively.

Similarly, the roots have the highest flavonoid content, with 50.81±2.5 mg QE g^{-1} of extract, followed by the three parts, bark, fibers and leaves, which are partially of the same order (Table 3). Moreover, the palm heart

Table 3

Total content of phenols, flavonoids and tannins in different parts of *C. humilis* extracts

Specification	TPC [mg GAE g^{-1} E]	TFC [mg QE g^{-1} E]	TTC [mg CE g^{-1} E]
Fibers	108.86±4.54^{a}	34.18±2.1^{a}	8.86±0.73^{a}
Barks	122.01±6.91^{a}	36.91±2.75^{a}	10.07±1.21^{a}
Root	151.09±2.83^{b}	50.81±2.5^{b}	7.83±0.35^{a}
Palm heart	79.12±2.17^{c}	19.35±1.56^{c}	–
Leaves	97.74±3.95^{a}	39.12±2.5^{ab}	1.97±0.69^{b}

The values are given as of triplets mean ± SEM, with similar letters in the different columns indicating significant ($P < 0.05$). GAE – gallic acid equivalent; QE – quercetin equivalent; CE – catechin equivalent; TPC – total phenolic content; TFC – total flavonoid content; TTC – total tannin content

had the lowest flavonoid content (19.35±1.56 mg QE per 1 g of extract). BENAHMED-BOUHAFSOUN (2018) found that flavonoid content of the leaves is twice as high as that of the roots, with respective contents of 40.7±0.53, and 20.04±0.62 mg EQ g^{-1} of extract, and reported a low content of flavonoid in the leaves (2.663±0.244 mg QE g^{-1} of extract)

As regards the tannin content, all parts are poor, with a value varying between 10.07±1.71, and 1.97±0.98 mg CE g^{-1} E, for the palm heart the amount of tannin was not detected. DJIPA et al. (2000) reported that the fibers contain 0.37 % of tannins

Antioxidant activity

The IC_{50} is a parameter that defines the concentration of antioxidants needed to reduce the initial concentrations of free radicals by 50%. The lower the IC_{50} value the greater the antioxidant action. The IC_{50} values of the alcoholic extracts, as well as those of the standards are shown in Table 4.

Table 4

IC_{50} results of different parts of *C. humilis* methanolic extracts and standards

Specification	DPPH IC_{50} [µg mL^{-1}]	ABTS IC_{50} [µg mL^{-1}]	FRAP EC_{50} [µg mL^{-1}]
Fibers	4.15±0.02^a	68.87±3.09^a	448.27±8.73^a
Barks	3.95±0.71^a	41.82±2.47^b	292.52±6.89^b
Roots	1.99±0.02^a	37.65±0.66^b	279.61±4.90^b
Palm heart	36.94±0.9^b	485.6±12.03^c	2315±10^c
Leaves	7.015±0.66^c	98.03±2.47^d	599.94±7.56^d
Ascorbic acid	1.91±0.04^a	–	45.8 ±1.9^e
Trolox	2.96±0.8^a	30.86±0.04^b	–

Data are presented as the mean of triplicates ± SEM, with numbers followed by similar letters in the same column not different with $P < 0.05$

Methanolic extracts of *C. humilis* fibers, bark, roots, leaves and heart of palm reduce free radicals with respective IC_{50} ranging from 1.99 to 36.94 µg mL^{-1} for DPPH and from 4.24 to 64.02 µg mL^{-1} for the cationic radical ABTS. The values follow the same order of predominance of the different parts studied. In addition, the roots have a very high activity which is practically similar to that of ascorbic acid which brings stability to DPPH with an IC_{50} of 1.91±0.04 µg mL^{-1}, this part has an IC_{50} equal to 1.99±0.02 µg mL^{-1} which is in part low compared to that of trolox 2.96±0.8 µg mL^{-1}. Similarly for the neutralization of ABTS, the roots

and barks retains the same IC_{50} order of 37.65±0.66 μg mL^{-1}, and 41.82±2.47 μg mL^{-1}, respectively. These values do not have a significant difference with that of the trolox which has an IC_{50} of 30.86±0.04 μg mL^{-1}, followed by fibers, which also have a remarkable value equal to 68.87±3.09 μg mL^{-1}.

Roots and barks have a capacity practically close to the reduction of ferric ions to ferrous ions with an EC_{50} of 279.61±4.90 and 292.52±6.89 μg mL^{-1}, respectively, then the fibers and finally the leaves which show a statistically significant difference, the latter two parts have a mean reduction compared to the others, but not the heart of the palm. According to these results, it is proven that ascorbic acid remains the most effective reducing agent with an EC_{50} of 45.8±1.9 μg mL^{-1} compared to the methanolic extracts of the different parts of *C. humilis* studied.

The results of the three antioxidant tests carried out show that the underground part has the best antioxidant activity, and the primary roots (the part which gathers the fibers, the barks and the heart of the stipe) are the most effectively used with low concentrations.

The polyphenols contained in the extracts obtained are probably responsible for the antioxidant activity. It can be noted that all the parts keep their order of predominance, the primary root has the highest levels of secondary metabolites, and this reflects the fact that it has the lowest IC_{50}, followed by bark, fibers and leaves. Nevertheless, the heart of the palm is the part that contains the lowest levels of active ingredients, reflecting its low reducing activity.

The results of the IC_{50} of the methanolic extract of the leaves show good antioxidant activity for DPPH, ABTS and FRAP with IC_{50} of 346.08±12.63 μg mL^{-1}, 593.23±9.80 μmol TE g^{-1} and 434.34±13.71 μmol TE g^{-1} extract, respectively, compared to the standard using BHT with an IC_{50} value of 462.24±0.03 μg mL^{-1} (Gonçalves et al. 2018).

The Antioxidant activity of *C. humilis* from leaves collected in Algeria, has already been tested by Benahmed (2013). The IC_{50} results reported IC_{50} of about 180.71±6.6 μg mL^{-1} in a DPPH test, comparing to the ascorbic acid 159.5±4.81 μg mL^{-1}.

Correlation Matrix

Table 5 shows the Pearson correlation, which allows us to analyze the relationship between the different variables tested in this study. Thus, Table 6 shows the *p*-values of the coefficients of the correlation matrix between all the variables. Based on the results obtained in the Table 5 and Table 6, we observed a significant positive correlation (*p*-value < 0.05)

between TFC and TPC ($r^2 = 0.898$). In addition, a highly significant positive correlation (p-value < 0.05) was detected between TPC and DPPH ($r^2 = 0.987$), TPC and ABTS ($r^2 = 0.956$), and FRAP ($r^2 = 0.936$), respectively. This indicates that the phenolic content of our extracts contributes to its ability to donate electrons to hydrogen. This reflects that the antioxidant capacity of our samples can be attributed to the existence of total phenols.

Table 5

Coefficient of the Pearson correlation matrix between the variables: TFC, TTC, TPC, ABTS, DPPH and FRAP of the different parts of *C. humilis*

Variables	TPC	TFC	TTC	$(1/IC_{50})$ to DPPH	$(1/IC_{50})$ to ABTS	$(1/IC_{50})$ to FRAP
TPC	**1**	–	–	–	–	–
TFC	0.898	1	–	–	–	–
TTC	0.740	0.559	**1**	–	–	–
$(1/IC_{50})$ to DPPH	**0.987**	**0.899**	0.699	**1**	–	–
$(1/IC_{50})$ to ABTS	**0.956**	0.839	0.856	**0.905**	**1**	–
$(1/IC_{50})$ to FRAP	**0.936**	0.837	0.877	**0.883**	**0.997**	**1**

Values in bold in Table 5 have a significant correlation at the level: alpha = 0.05

Table 6

p-values of the correlation matrix coefficient between all variables

Variables	TPC	TFC	TTC	$(1/IC_{50})$ to DPPH	$(1/IC_{50})$ to ABTS	$(1/IC_{50})$ to FRAP
TPC	**0**	–	–	–	–	–
TFC	**0.038**	0	–	–	–	–
TTC	0.153	0.327	**0**	–	–	–
$(1/IC_{50})$ to DPPH	**0.002**	**0.038**	0.189	**0**	–	–
$(1/IC_{50})$ to ABTS	**0.011**	0.075	0.064	**0.034**	**0**	–
$(1/IC_{50})$ to FRAP	**0.019**	0.077	0.051	**0.047**	**0.000**	**0**

Values in bold in Table 6 have a significant correlation at the level: alpha = 0.05

The positive linear correlation between the phenolic content and the antiradical capacity has also been reported by several authors (GUETTAF et al. 2016, AMRI et al. 2015). There was also a significant ($p < 0.05$) positive correlation between TFC and DPPH ($r^2 = 0.899$). However, tannins do

not contribute much to the antioxidant activities tested; hence, its correlation coefficients are relatively low. Contrary to polyphenols and flavonoids which contribute strongly to this bioactivity. Therefore, these strong correlations indicate that the bioactive agents, which contribute to the iron-reducing power by FRAP, are themselves the source of the free radical scavenging power by DPPH and ABTS assays. Furthermore, the correlation matrix showed a strong correlation between the three antioxidant tests, between DPPH and ABTS (r^2 = 0.905), and between DPPH and FRAP (r^2 = 0.883), and between FRAP and ABTS (r^2 = 0.997) with (p-value < 0.05). The positive correlation between the three antioxidant tests is hard to the strong presence of total phenols, the results found are very promising, and this allows us to say that our samples, as well as our chosen plant can be considered from the best natural antioxidants.

Principal Component Analysis (PCA)

The results of polyphenol, flavonoid, and tannin contents, and the antioxidant activity DPPH, ABTS and FRAP are considered as variables. They are projected by PCA on the F1–F2 factorial planes (Figure 1).

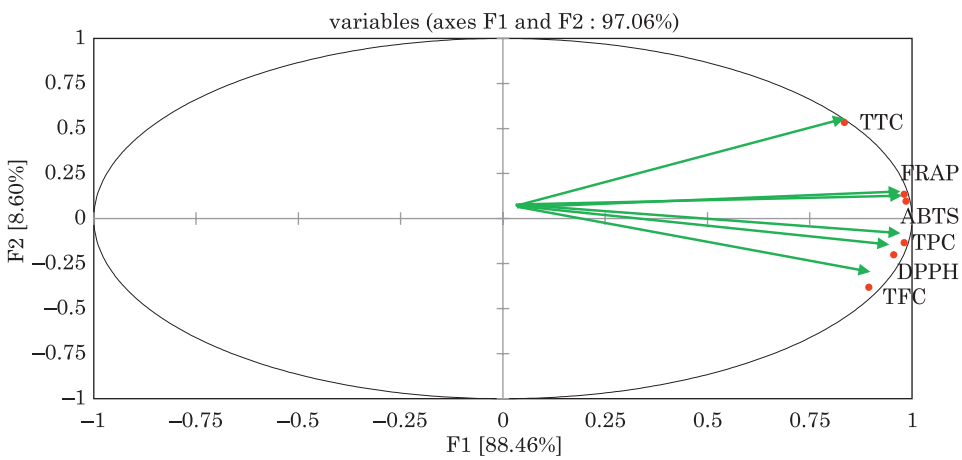

Fig. 1. Correlation circles between all variables

The first major component F1 explains 88.46% of the total information and the second major component F2 presents 8.60%. The cumulative percentage of the first two major components being 97.06 %, its linear combination represents the variables, as it is greater than 50%. Therefore, the first two axes are appropriate to explain the information as a whole. Figure 1 shows the plane formed by the F1 and F2 axes, which gives the correlation

between the variables. The F1 and F2 axes consist mainly of the positive correlation between TTC, TFC, TPC, and between DPPH, ABTS and FRAP tests.

The theoretical data in Figure 2 show the existence of three groups. The first group is formed by an extract called roots, which is characterized by the highest contents of total polyphenols and flavonoids, as well as by its very high antioxidant efficiency by DPPH, ABTS and FRAP.

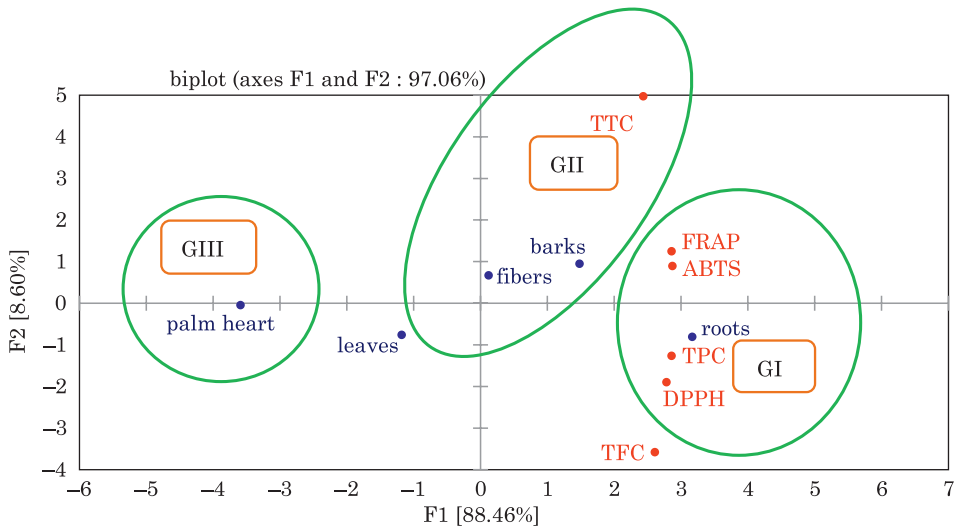

Fig. 2. Correlation variables-parts (GI – group I; GII – group II; GIII – group III)

The second group consists of bark, fiber and leaves, which are characterized by a higher TTC value. In addition, they have average levels of TPC and TFC. Likewise, their antiradical activities (DPPH, ABTS and FRAP) are close to those of the first group. It is also observed that the roots have an average TTC content. The rest of the studied part (heart palm) belongs to the 3rd group, which is far from the other two groups, reflecting that the heart palm has very low contents of the studied secondary metabolites compared to the other parts. Thus, their tested inhibitory actions are also low.

The analysis of the data by PCA, showed the presence of a very high positive correlation, between phenolic compounds and antioxidant activity by DPPH, ABTS and FRAP. This showed that in our extracts, the bioactive compounds, which provide the free radical scavenging activity, as well as the reduction of ferric ions to ferrous ions, by DPPH, ABTS and FRAP tests, respectively.

Hierarchical clustering analysis (HCA)

According to HCA, the extracts were grouped by Euclidean squared method and Wards method to identify the (dis)similarity measure. The HCA was used to evaluate the correlation between the extracts, as well as to present the similarities of the 5 tested samples based on the data of bioactive agent contents and antioxidant activity, as shown in the dendrogram in Figure 3. According to the bioactive molecules, the 5 extracts were classified into three clusters.

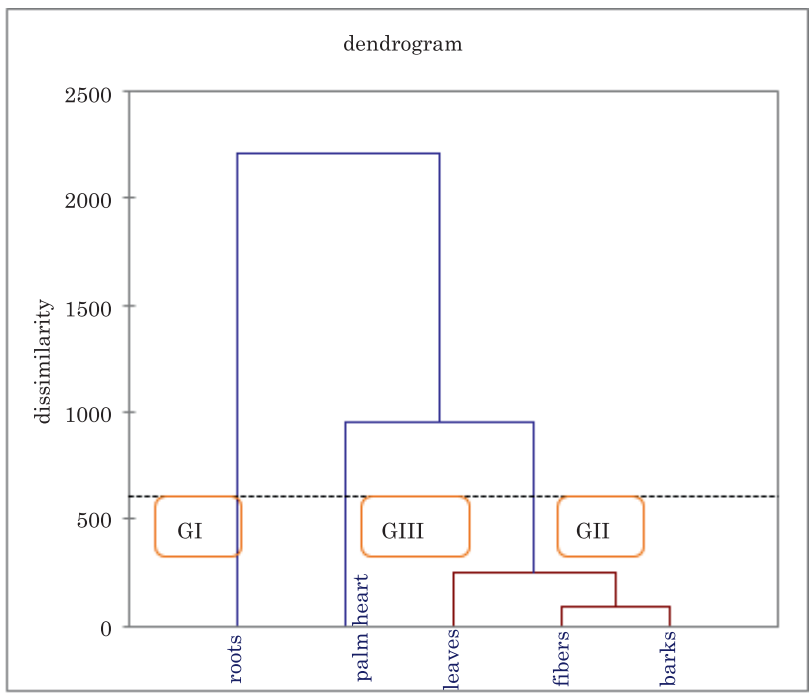

Fig. 3. Dendrogram of the extracts tested founded by HCA, using on bioactive and their antioxidant capacities (GI – cluster I; GII – cluster II; GIII – cluster III)

Cluster II, contains a single extract of the roots, and which represented 20% of the total extracts. This extract had the highest value of TPC, TFC, and TTC reached about: 151.09±2.83 mg GAE g^{-1} E, 50.81±2.5 mg QE g^{-1} E, and 7.83±0.35 mg CE g^{-1} E, respectively. This allows it to be characterized by a strong antioxidant power by DPPH, ABTS and FRAP tests.

Cluster II, is formed by 3 extracts named respectively leaves, fibers, and barks, representing 60% of the total extracts, characterized by a medium mean values of TPC, TFC, and TTC (see Table 3), as well as by an average antioxidant capacity compared to that of cluster I.

Cluster III contained an extract, called palm heart, representing 20% of the total extracts, characterized by low values of secondary metabolites, TPC (79.12±2.17 mg GAE g^{-1} extract), and TFC (19.35±1.56 mg QE g^{-1} extract), respectively. As well as lower antioxidant power. These results are consistent with the data obtained by PCA, in which the distribution of all extracts on the score plot indicates an identical trend.

Conclusion

Phytochemical tests performed on the five parts reveal the existence of interesting chemical families: tannins, flavonoids, saponins, coumarins and terpenes. The quantitative analyses carried out show that the methanolic extracts of the roots, barks, fibers and leaves of *C. humilis* are rich in phenols, flavonoids and tannins. Given the results obtained in this study, it can be concluded that the three methods used can quantify the antioxidant activity of the 5 part of the plant species, this activity can be attributed to the high existence of phenolic compounds. The results of the various tests carried out are promising for a possible valorization of *C. humilis* as a bioresource in the therapeutic field.

Conflicts of Interest

Authors declare that there are no conflicts of interest regarding the publication of this paper.

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