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Biological Properties of *Chamaerops humilis* L. – Antioxidant and Antibacterial Activities of Leaf, Fruit and Pulp Extracts

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ABSTRACT

This work aimed to evaluate the polyphenol and flavonoid composition, antioxidant and antibacterial activity of leaf, fruit and pulp extracts of *Chamaerops humilis* L. Dry extracts of leaves, fruits and pulp were prepared by ultrasonic extraction and examined as potential sources of phenolic compounds and flavonoids. Different methods were used to evaluate the antioxidant activity of the extracts, including DPPH free radical scavenging assay and total antioxidant capacity (TAC). The total polyphenol content (TPC) and total flavonoid content (TFC) of the tested extracts were examined by the Folin-Ciocalteu and aluminium chloride ($AlCl_3$) methods, respectively. The antibacterial activity of leaf, fruit and pulp extracts against a collection of bacterial strains was evaluated using various *in vitro* methods, including well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results suggest that the leaf, the fruit and the pulp extracts have good potential as sources of bioactive compounds, the TPC and TFC of leaves were 116.209 ± 1.58 and 2.313 ± 0.02 mg GAE/g d.w, respectively. The TPC and TFC were 78.621 ± 1.06 and 0.425± 0.02 mg GAE/g dry weight in fruits, respectively. The best ability to trap DPPH radical was observed in the leaf extract (IC50 = 4.006 ± 0.36 mg/ml d.w); also, this extract revealed a better total antioxidant capacity of 119.702 ± 1.59 mg AGE/g dry weight. Regarding antibacterial activity, the leaves showed an important antibacterial activity against the tested microorganisms with MIC ranging from 0.195 mg/ml to 3.125 mg/ml and with an inhibition diameter ranging from 12.03 ± 0.2 mm to 16.26 ± 0.03 mm Furthermore, a strong correlation was observed between phytochemical parameters (TPC and TFC) and biological activities (antioxidant and antimicrobial activities). These results revealed that leaves, fruits and pulp extracts of *C. humilis* are a good source of bioactive compounds with potent antioxidant and antibacterial potentials. Therefore, they can be a new and alternative source of products for medical and industrial applications.

Keywords: *Chamaerops humilis* L., chemical composition, antioxidant activity, antibacterial activity.

INTRODUCTION

Chamaerops humilis L. is considered among the medicinal plants largely used in traditional medicine. It is known for its antibacterial and antioxidant activities, as it constitutes a promising source of bioactive molecules that play a major role in treating bacterial infections (El Cadi et al., 2021; Blumenthal et al., 1998). *Chamaerops humilis* L. is characterized by its history in popular Moroccan medicine; it has been used because of its nutritional and pharmaceutical properties (Emad et al., 2021). In addition, the fruits of this species are known to be rich in nutritional elements; also, they are generally astringent due to their high tannin content (Bnouham et al., 2010; Bouhafsoun et al., 2019). It is abundant in southern Europe and North Africa (Freitag, 1971). This species is reported to have diverse biological properties, such as antidiabetic, antimicrobial, anti-inflammatory, anabolic, antilithic, and diuretic effects (Hasnaoui et al., 2016; Benmehdi et al.,

2011). The main phytochemical compounds that characterize this plant are polyphenols, flavonoids, tannins, terpenoids, and saponins, which have contributed to the control of various human physiological processes and protection against oxidative stresses, inflammation, microbes and cancer (El-Beltagi et al., 2018; Faten, 2009).

Free radicals contribute to their role in triggering chronic degenerative diseases, including cancer, autoimmune diseases, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases and aging in general (Seyfried, 2007; Aruoma, 2003; Surh et al., 2001). As a result of these facts, synthetic and natural products have received increased attention in biological research, medicine, and pharmacy due to their antioxidant activity (Seyfried, 2007 ; Goodman et al., 2011).

The control of bacterial infections is realized mainly by the use of antibiotics; these means of treatment are sometimes inappropriate because of the resistance of microorganisms to antibiotics, hence the interest to direct studies toward alternative medicine, which constitute a source of new natural molecules with important antimicrobial properties (Billing & Sherman, 1998).

The present study aimed to evaluate the scientific corpus of the therapeutic effects of *humilis.* For this purpose, a quantification of the chemical compounds present in this plant and the implementation of its antioxidant and antibacterial power were evaluated. The results of this study will be a source of information on this plant for better valorization and to maintain its sustainable development.

MATERIALS AND METHODS

Sample preparation

The parts of *humilis* (leaves, fruits and pulp) were collected in 2020. The plant was identified by botanist Amina Bari, under voucher number CH185181S1 (FSDM, USMBA, Fez, Morocco). The plant material was dried in the oven at 40°C until a constant weight was obtained, then ground to powder using a hand blender and sieved through a sieve of diameter < 2 mm.

Extraction procedure

The extraction was performed by the sonication technique with the selected extraction solvent: the solvent composed of ethanol, water and methanol for the leaf part, the hydroethanol solvent for the fruit part and the hydromethanol solvent for the pulp part. The obtained solution was concentrated in the rotavapour, until a solid was obtained, and stored at -20°C until use.

Phytochemical tests

Determination of the total phenolic content

The total polyphenol content of hydroalcoholic extracts was determined by the Folin – Ciocalteu method described by Singleton et al., (1999) with some modifications. Firstly, 50 µL of the extract was mixed with 450 µL of Folin – Ciocalteu reagent (0.2 N); after 5 min, 450 µL of Na_2CO_3 solution (75 g L^{-1}) was added to the mixture, then the whole was incubated in the dark for 2 h at room temperature. The absorbance was measured at 760 nm in a Jenway 6505 UV/visible scanning spectrophotometer. The results are expressed as mg gallic acid equivalent (GAE) g^{-1} dry weight

Determination of the total flavonoid content

The amounts of total flavonoids in the extracts were determined according to the method described by (Miguel et *al*., 2010) with minor modification. Initially, 200 µL of the extract was mixed with 100 µL of $AlCl_3$ (10%), and after vortexing, 700 µL of distilled water was added. The mixture was incubated for 40 min in the dark at room temperature. The absorbance was measured at 420 nm. Total flavonoid content was calculated as mg quercetin equivalents (QE) per gram (g) of dry weight.

Biological activities

Antioxidant activities

DPPH free radical-scavenging activity

The free radical scavenging activity of all extracts was determined by the method described by (Aazza et al., 2011). Briefly, 50 µl of extract from the different dilutions were added to 950 µl of a methanolic solution of DPPH (60 µM). After 60 min of incubation at room temperature, the absorbance was measured at 517 nm. The percentage of inhibition of the free radical scavenging activity of the extracts was calculated as follows:

$$\% \text{ Inhibition} = [(\text{Abs control} - \text{Abs sample} / \text{Abs control}) \cdot 100] \quad (1)$$

Total antioxidant capacity

The total antioxidant capacity (TAC) of the extracts was evaluated by the formation of the green phosphomolybdenum complex, according to (Prieto et al., 1999). An amount of 50 µL of the sample was mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes. The mixture was incubated in a water bath at 95°C for 90 min. The test tubes were cooled to room temperature, and the mixture absorbance was measured at 695 nm against a blank in a Jenway 6505 UV/visible scanning spectrophotometer. The calibration curve for the aqueous ascorbic acid solution included a concentration series of 5.0 to 0.0039 mg ml^{-1}. The results showed (antioxidant activity in ascorbic acid equivalents) are mean values expressed as (g) ascorbic acid equivalent (EAA) g^{-1} dry weight.

Antimicrobial activities

The conducted investigation consisted in evaluating the antimicrobial effect of the extracts of leaves, fruits, and pulp of *Chamaerops humilis* L. by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), as well as the diameter of the zone of inhibition (DI) using the microplate method and the well method.

Bacterial strains and inoculum preparation.

The microorganisms used in this study were Gram positive bacteria, *Bacillus subtilis* IL-P1428B, *Staphylococcus aureus* CIP543154, and Gram negative bacteria, *Pseudomonas aeruginosa* ATCC27653, *Escherichia coli* CIP5412 which were collected from the American Type Culture Collection (CATC) and the Institut Pasteur Collection (CIP). Bacterial growth was realized at 37°C in LB medium. The bacterial suspension (inocula) was prepared by picking a few colonies with a sterile loop and transferring them into 10 ml of physiological saline (0.9% NaCl). The resulting bacterial suspension was homogenized using the vortex. The density was adjusted to saturation of 0.5 McFarland, which corresponds to an optical density of 0.08–0.10 measured at a wavelength of 625 nm. The final concentration of the inoculum was around 108 CFU/ mL (Dimitrijevic et al., 2012).

Agar well diffusion method

The antimicrobial power of leaf, fruit and pulp extracts of *Chamaerops humilis* L. was examined by the well diffusion method (Hindi, 2013; Clinical and Laboratory Standards Institute, 2012). The bacterias were subjected to antibacterial effect according to the method mentioned above. Approximately 50 ml of LB medium was poured into Petri dishes; each Petri dish was inoculated with bacterial inoculum consisting of 0.5 McFarland (1–2)·10^8 CFU/ mL, which was prepared in physiological saline buffer, 100 µL of leaf, fruit and pulp extract of the studied plant was placed in wells formed in the agar of each Petri dish. Negative control wells were filled with sterile physiological water. After 24 h of incubation at 37°C, the diameter of the inhibition zones was measured in mm. Each experiment was performed in triplicate.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is the lowest concentration of the extract that is able to inhibit bacterial growth, Following the NCCLS method (Clinical and Laboratory Standards Institute. 2012). The MIC of each extract was determined. This technique was performed by a series of dilutions of the leaf, fruit, and pulp extracts of the plant studied, with concentrations ranging from 0.048 and 100 mg/mL, 50 µL of each concentration was mixed in a 96-well plate with 50 µL of LB medium and 50 µL of bacterial inoculums (5·105 CFU/mL). In parallel, a positive control which contains 50 µL of bacterial inoculums plus 50 µL of LB medium, and a negative control which consists 50 µL of L medium, were prepared. After incubating the microplates at 37°C, 15 µl of resazurin developer was added to each well and incubated for 30 min. Microbial growth is revealed by the absence of purple color after the addition of resazurin.

Minimal bactericidal concentration (MBC)

MBC is defined as the low concentration necessary to kill 99.9% of the inoculated bacteria (Dimitrijevic et *al*., 2012). In order to determine the MBC, each of the present concentrations (> or = (MIC) was sub-cultured onto LB agar and incubated at 37°C for 24 h. In fact, if the MBC/ MIC=1-2, the antibacterial effect is bactericidal; if the MBC/MIC = 4 to 16, it is a bacteriostatic effect (Berche et al., 1991).

Statistical analysis

All experiments were performed three times using triplicate samples. The data are presented

as mean ± standard deviationff. The differences were evaluated using an analysis of variance (ANOVA) with a $p < 0.05$ significance level. All statistical analyses were performed using SPSS (version 25, IBM SPSS Statistics 25), and when the effect was revealed to be significant, the Tukey test was performed for means separation at a significance level of $P < 0.05$. Pearson correlation test and PCA were performed using XLSTAT 2014.5.03 software.

RESULTS

Bioactive compounds

Total polyphenol content

The results represented in Table 1 highlight the variation of the total polyphenol content at the level of the plant parts (leaves, fruits and pulp). On the one hand, the optimized extract of leaves is the richest in polyphenols with a rate of 116, 209±1.58 mg GAE/g dry weight. The fruit extract also has a significant level of polyphenols equal to 78.621±1.06 mg GAE/g dry weight. On the other hand, the pulp extract accumulates a lower concentration than the other studied extracts, equal to 15.848±0.27 mg GAE/g dry weight. The statistical comparison showed that the total phenolic content of different samples was significatively different ($p < 0.05$).

Total flavonoid content

The results of the flavonoid content of the three optimized extracts of the studied parts (leaves, fruits and pulp) are reported in Table 1. The leaf extract recorded a high flavonoid concentration (2.313±0.02 mg GAE/g dry weight). The two extracts represent the lowest flavonoid contents, which are in the order of (0. 425±0.0227 and 0, 208±0.01 mg GAE/g dry weight) for the fruit and the pulp, respectively. Statistical analysis of the obtained results showed highly significant differences ($P \leq 0.01$) between leaf, fruit and pulp extracts ($F = 6.919$; df*=1). In turn, there was no significant difference between fruit and pulp extract ($p \geq 0.05$), ($F = 4.965$; df* =1).

Biological activities

Evaluation of the antioxidant activity

Antioxidant compounds play a major role in preventing several diseases, including neurodegenerative diseases, because they neutralize the oxidative stress that produces free radicals (Tabet, 2006). The search for antioxidants from natural sources is of global interest, as they are less toxic and more potent than synthetic antioxidants (Abdel-Hameed et al., 2014). Several medicinal plants have been studied as potential sources of natural antioxidants (Nile et al.; Suluvoy & Grace, 2017). In the conducted study, the antioxidant activity of three extracts of *Chamaerops humilis* L. (leaves, fruit and pulp). was studied using DPPH and CAT tests; the results are presented in Table 2.

The antioxidant capacity of the extracts of different studied parts was determined from the IC50, which is the necessary concentration to reduce 50% of free radicals. The values of IC_{50} are inversely related to the antioxidant capacity of a compound; the antioxidant power is proportional to IC50; the lower the value of IC_{50}, the stronger the antioxidant power of the extract tested (Hebi & Eddouks, 2016). The IC50 values obtained from the studied parts of the extracts are represented in Table 2. The IC50 recorded for ascorbic acid (3.191±0.07 mg/ml) used as a reference molecule is higher than the different studied extracts. The leaf and fruit parts have an IC50 of about (4.006±0.36 and 4.967±0.41 mg/ml), respectively. In contrast, the pulp part records a higher concentration (5.526±0.29). Thus, the leaves present the most active part. The statistical treatment of the IC50 values revealed highly significant differences between the different studied parts ($p \leq 0.001$), ($F = 4.351$; df* =1). The results obtained showed that the leaf extract revealed

Table 1. Total phenolic and flavonoid determining contents of *Chamaerops humilis* L. extracts

C. humilis	Polyphenols mg GAE / g d.w	Flavonoides mg GAE / g d.w
Leaves	116. 209 ± 1.58 [a]	2.313 ± 0. 02 [a]
Fruit	78. 621 ± 1.06 [b]	0. 425 ± 0.02 [b]
Pulp	15. 848 ± 0.27 [c]	0. 208 ± 0.01 [b]

Note: values are expressed as means ± SD (n = 3). Values followed by different letters are significantly different at $p < 0.05$; GAE = gallic acid; d.w = dry weight, df* = degrees of freedom.

Table 2. Antioxidant activity of different parts of *Chamaerops humilis* L. determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and total antioxidant capacity (CAT) assays

Plant part	DPPH (IC50, mg/ml d.w)	TAC (mg AGE/g d.w)
Leaves	4.006 ± 0.36 [c]	119.702 ± 1.59[a]
Fruit	4.967 ± 0.41 [b]	83.256 ± 1.79[b]
Pulp	5.526 ± 0.29 [a]	18.887 ± 0.42[c]

Note: values are expressed as means ± SD (n=3). Values with different letters are significantly different at $p < 0.05$. Reference in DPPH assay, Ascorbic acid : IC_{50} = 3.191±0.07 mg/ ml; d.w = dry weight; TAC = total antioxidant capacity; DPPH = 2,2-diphenyl-1-picrylhydrazyl.

a better total antioxidant activity than the fruit and pulp extracts. The antioxidant concentration in the leaf extract is (119.702 mg AGE/g dry weight). However, the pulp extract has the lowest antioxidant concentration (18.887 mg AGE/g dry weight). Statistical analysis of the results reveals a highly significant difference between the three studied extracts ($F= 4.60$; df* =1 ; $P \leq 0.001$).

Evaluation of the Antimicrobial activity

The bioactive substances of medicinal plants have a therapeutic capacity against microbes. Chemical compounds, including flavonoids, saponins, alkaloids, tannins and terpenoids, present strong antimicrobial activity against fungi and bacteria (Oladeji, 2016). Table 3 presents the sensitivity of the tested bacterial strains to the extracts of different parts of the studied plant (leaves, fruits and pulp) by measuring the inhibition zones in mm.

The inhibitory capacity of the evaluated leaf and fruit extract revealed a positive effect against the tested bacterial strains with a range of inhibition zones between (9.01±0.02 and 16.26±0.03 mm). In contrast, the pulp extract had no effect, but only on the bacterial strain *Bacillus subtilis* the inhibition diameter is (11.1±0.01 mm). The leaf extract was the most effective against all strains tested as it inhibited the growth of all isolates with an inhibition zone ranging from (12.03±0.2 mm) for *Escherichia coli* to (16.26±0.03 mm) for *Bacillus subtilis*. The lowest inhibitory effect was registered for the fruit part against the bacterium *Pseudomonas aeroginosa* with an inhibition diameter of (9.01±0.02 mm).

Table 4 presents the results of minimum inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC) of each part of *Chamaerops humilis* L. against all studied Gram-positive and negative isolates, as well as the type of effect that characterizes each extract. Evaluation of the antibacterial activity of extracts of different parts of *Chamaerops humilis* L. (leaves, fruits and pulp) indicated a large spectral antibacterial activity.

The MIC values of the leaves, fruits and pulp ranged from 0.195 mg /mL to 25 mg/mL on four bacterial strains and the MBC values ranged from 0.39 mg /mL to 50 mg/mL. The antibacterial activity results revealed that the highest MIC of pulp extract (25 mg/mL) was observed against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. However, the lowest MIC of leaf extract (0.195 mg/ml) was recorded against *Bacillus subtilis*. Similarly, the fruit extract has an antimicrobial power against the different strains studied. The lowest MIC was recorded against Staphylococcus aureus at a concentration of 3.125 mg/ml, and the highest MBC was obtained against *Bacillus subtilis* at a concentration of 50 mg/ml.Therefore, it can be argued that the three extracts of leaves, fruit and pulp have an antimicrobial effect against the different bacterial strains studied. Among the bacterial strains tested, *Bacillus subtilis* was the most sensitive to leaf and pulp extracts, while the *Staphylococcus aureus* strain was equally sensitive to the fruit extract.

Table 3. Diameters of inhibition zones (DI) of different parts of *Chamaerops humilis* L.

C. humilis (100 mg/mL)	*Pseudomonas aeroginosa* (ATCC27653)	*Escherichia coli* (CIP5412)	*Staphylococcus aureus* (CIP543154)	*Bacillus subtilis* (ILP1428B)
Leaves	13.06 ± 0.04	12.03 ± 0.2	14.09 ± 0.02	16.26 ± 0.03
Fruit	9.01 ± 0.02	11.19 ± 0.05	13.2 ± 0.02	14.14 ± 0.04
Pulp	---	---	---	11.1 ± 0.01

Note: DI – diameters of inhibition zones.

In contrast, the *Escherichia coli* strain showed a very marked resistance to the samples examined.

Table 4 shows the MBC values of the three studied extracts. In fact, the highest MBC was 0.39 mg/ml, shown against *Bacillus subtilis* by the leaf extract. For the fruit extract, the most interesting MBC was against *Staphylococcus aureus*, while the MBC of *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa* were 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml, respectively. The pulp extract had an MBC of 3.125 mg/ml against *Bacillus subtilis* and 25 mg/ml against *Pseudomonas aeruginosa*. However, against *Staphylococcus aureus* and *Escherichia coli*, the MBC was 50 mg/ml. According to the MBC/MIC ratio, it appears that the extracts of different parts of *C. humilis* had a bactericidal activity on the studied strains.

Correlation

The correlations between phytochemical parameters and antioxidant and antimicrobial activities are presented in Table 5. Almost all correlations are highly significant ($r > 0.900$; $p < 0.0001$). A strong positive correlation was registered between bioactive compounds (TPC, TFC), TAC and DI of *P. aeruginosa*, *E. coli* and *S. aureus*. Similarly, TPC and MIC of *E.coli* had a strong correlation ($r = 0.969$; $p = 0.001$). However, a negative correlation was observed between TPC, TFC, TAC and IC50% DPPH, MIC of *P. aeruginosa*, *E. coli* and *S. aureus*. This suggests that this group of chemical compounds can play an interesting role in the biological activities of the extracts of the studied plant parts (leaves, fruits and pulp *Chamaerops humilis* L).

Multivariate analysis

In order to highlight the relationships between the presence of phenolic compounds (polyphenols, flavonoids) and the antioxidant activity of the studied extracts by chemical tests DPPH and TAC, as well as the antibacterial activity by DI and MIC. It is essential to use all the statistical tools, such as the PCA, which is an effective way to determine the relationship between variables and similarities between parts (Forina, et *al*., 1987). Figure 1 shows the principal component analysis (PCA), carried out on all the parameters studied, taking into account the three organs of the plant. In the present study, the first two principal components (F1 and F2) expressed the maximum information contained in the initial data matrix (87.89% and 12.11%, respectively). The first principal component (PCA), which aggregates most of the information, was positively correlated with the TFC and TPC parameters and the diameter of inhibition zone results. In contrast, a negative correlation was noticed between the same main component and the antibacterial and antioxidant activities. PCA allowed the formation of three distinguished groups; the first one

Table 4. MICs and MBCs values of *Chamaerops humilis* L.samples generated against different bacterial strains

C. humilis		*Pseudomonas aeruginosa* (ATCC27653)	*Escherichia coli* (CIP5412)	*Staphylococcus aureus* (CIP543154)	*Bacillus subtilis* (ILP1428B)
Leaves	CMI (mg/ml)	3.125	6.25	3.125	0.195
	CMB (mg/ml)	6.25	12.5	3.125	0.39
	CMB/ CMI	2	2	1	2
	Effet type	Bactricidal	Bactricidal	Bactricidal	Bactricidal

Note: CMI – minimum inhibitory concentration; CMB – minimum bactericide concentration.

Table 5. Pearson correlation matrix of phytochemical parameters, Diameter of inhibition (DI) and MICs of leaf, fruit and pulp extracts

	DPPH (IC50)	DI *P. aeruginosa*	DI *E. coli*	DI *S. aureus*	DI *B. subtilis*	CMI *P. aeruginosa*	CMI *E. coli*	CMI *S. aureus*	CMI *B. subtilis*
TPC	- 0.893**	0.964***	0.890**	0.888**	0.979***	- 0.918***	0.969***	-0.864**	-0.072
TFC	-0.814**	0.805**	0.641	0.637	0.865**	- 0.693*	- 0.820**	-0.593	-0.481
TAC	- 0.930***	0.998***	0.953***	0.952***	0.998***	- 0.972***	-0.999***	-0.933***	0.054

Note: ***Correlation is significant at the 0.001 level, **correlation is significant at the 0.01 level, *correlation is significant at the 0.05 level.

is constituted by the leaf part, which is characterized by high polyphenol and flavonoid content and, consequently, high antioxidant and antibacterial activity. This group is located on the negative side of the F2 axis. The fruit part is located on the positive side of the F2 axis and is characterized by the DPPH antioxidant and antibacterial activity. However, the pulp part is present on the negative side of the F1 axis, and characterized only by the antibacterial power.

DISCUSSION

Total phenolic compounds are a group of plant compounds of importance in the biotechnological and industrial sectors and are the main contributors to different biological activities of plants (Robards et al., 1999). Phytochemical analysis of leaf, fruit and pulp extracts of *Chamaerops humilis* L, by quantitative determination of polyphenols, revealed that the highest polyphenol content was registered in the leaf and fruit extract, whereas the lowest was in the pulp extract. This important presence of polyphenols can be explained by the fact that the leaves and fruits are more exposed to UV rays than the pulp part. The obtained results are in accordance with (Gonçalves et al., 2018) who reported that the highest phenolic content was found in the leaf extract, and the lowest in the pulp extract. The results of the polyphenol content of the fruit of this plant is clearly higher (78,621 mg/g dry matter) than the ones found by (Mohamed et al., 2010) (64.9 GAE/g d.w). In comparison with other studies, the obtained results of polyphenol content at the leaf level are high compared to the values found by (Khoudali et al., 2014) (99.8 mg EAG/g (sample). In addition, it was noted that the extract of the analyzed leaves shows a polyphenol content superior to that found by (Bengag, 2009) (25.2 µg EAG/mg extract) and lower than the one obtained by (Coelho et al., 2017) (153.1 µg EAG/mg extract). Flavonoids are largely distributed in the plant world and they also have different bioactive properties. A study carried out on *Chamaerops humilis* L. showed that the highest flavonoid content was found in the leaf extract, and the lowest in the pulp extract (Gonçalves et al., 2018), the same results were obtained in authors' own work. The gathered results agree with those obtained in the literature, the highest flavonoid contents in the leaves of *Chamaerops humilis* L. (Coelho et al., 2017). The high presence of this chemical compound in the aerial parts (leaves and fruits) is explained by the fact that flavonoids ensure the protection of plant tissues against the effects of UV radiation since they absorb these radiations

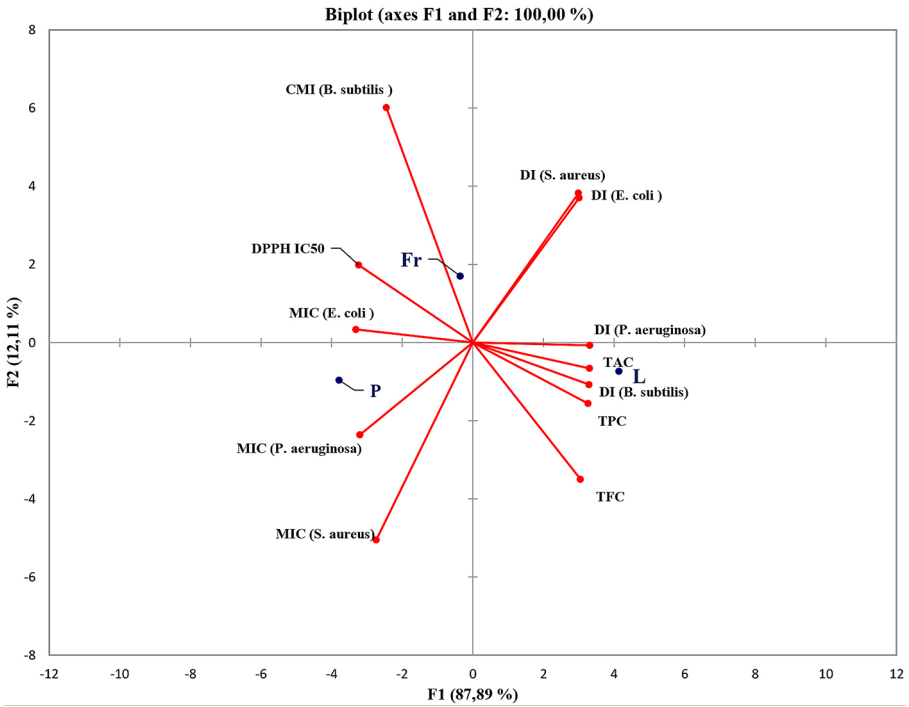

Figure 1. Principal component analysis (PCA) of leaves (L), fruit (Fr) and pulp (P) of the studied plant using the evaluated parameters: TPC, TFC, IC_{50} DPPH, TAC, DI and MIC

and act as solar filters, hence the high presence of flavonoids in the plant parts exposed to the sun (leaves and fruits) than those in the shade (pulp) (Acherrat, 2016). In the presented study, flavonoids were found only in minimal amounts (0, 425 mg/g d.w.) in the fruits of this plant compared to the work conducted by (Mohamed et al., 2010) which showed an important amount of flavonoids in the fruit extract (46.28 mg/g d.w). The difference that was found between the obtained results and those presented by the authors is generally due to several factors, including, the extraction method, the period of collection of different parts of the plant (leaves, fruit and pulp) and the stage of development of the plant (Belhaoues, 2018). This difference can also be explained by the intrinsic physiological and morphological characteristics of the plant and the growing conditions. All these factors taken together may influence the performance of the three parts of the plant with respect to phytochemical content.

Concerning the antioxidant capacity, it is recommended to base on multiple antioxidant test models (Alam et al., 2013 ; Singh & Singh, 2008). In the present study, the studied extracts were examined by two different methods: DPPH and TAC. The DPPH test is a rapid, easy, economical and largely used method. It is based on the reduction of the violet radical of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH-) to the yellow non-radical diphenylpicrylhydrazine (DPPH-H) in the presence of the antioxidant hydrogenator (Singh & Singh, 2008; Pisoschi & Negulescu. 2011). The total antioxidant test (TAC) is carried out at acidic pH. It is based on the reduction of Mo(VI) to Mo(V) by the analyzed sample and the thereafter formation of a green phosphate/Mo(V) complex (Prieto et al., 1999). The conducted study showed that the leaf extracts of Chamaerops humilis L. revealed strong DPPH piecing activity, and the fruit extract showed significant antioxidant activity. These results indicate that these extracts notably affect the trapping of free radicals. The obtained results are in accord with (Lantto et al., 2009; Wang et al., 2009). (Eldahshan et al., 2009) also showed that aqueous-ethanolic extract of this plant's leaves seems to capture reactive oxygen species fully. Similarly, the leaf extracts were identified as having the highest antioxidant activity with the TAC test, followed by the fruit and pulp parts. The existence of great differences between the antioxidant capacity in the parts of the plant can be due to their richness in phenolic compounds (Simonne et al., 1993). He noted that phenolics are responsible for the antioxidant activity of plant materials (Hsu et al., 2006 ; Rice-Evans et al., 1996). Studies have suggested the role of phenolic compounds as the principal sources of natural antioxidants in plant foods (Hagerman et al., 1998). In the conducted study, the fruit extract showed potent antioxidant activity. This high free radical scavenging activity could be due to its richness in phenols and flavonoids (Chua et al., 2013). The results obtained are in agreement with (Cook et al., 1998) having reported that the aqueous extract of the fruit part of *Chamaerops humilis* L. revealed antioxidant activity ; this is generally due to the substantial quality of their phenolic. It is well known that the plants rich in flavonoids are a good source of antioxidants (Bouterfas et al., 2016 ; Djeridane et *al*., 2006).

According to scientific research, several studies have reported many active functions of *Chamaerops humilis* L., including antibacterial activities (Belhaoues, 2018; Medjati, 2014). The present work aimed to evaluate the antibacterial power of leaf, fruit and pulp extracts of the studied plant, as well as to predict the sensitivity and resistance of Gram- and Gram+ bacteria to the studied extracts. The results of the conducted study showed that the extracts of leaves, fruit and pulp are active to various degrees and revealed an interesting antibacterial activity. The phenolic composition could influence the antibacterial effect of these extracts; indeed, these molecules are gifted with an important antibacterial power on the different gram-positive and gram-negative bacterial strains (Olivier et al., 2017; Al Akeel et al., 2014; Martins, et al., 2013). In addition, (Cowan, 1999) reported that several classes of polyphenols, such as phenolic acids and flavonoids, serve as plant defense mechanisms against pathogenic microorganisms. Among the bacterial strains tested, *Bacillus subtilis* was the most sensitive to plant leaf and pulp extracts. The obtained results do not concord with those obtained by (Acherrat, 2016), which revealed that the strain *Pseudomonas aeruginosa* having a sensitivity towards butanolic extracts of the two parts, leaves and stipe heart. Chaturvedi and Taleb-Contini (Chaturvedi et al., 2010; Taleb-Contini et al., 2003) pointed out that all flavonoid extracts have important antimicrobial activity against all tested bacterial strains. The antibacterial activity results also revealed that *Staphylococcus* aureus showed high sensitivity to *Chamaerops humilis* L.

fruit extract. This could explain the richness of the fruit extract in polyohenols and flavonoids and its efficiency against the studied strains. The obtained results are coherent with those of (Emad et al., 2021) found that this plant's methanolic fruit extract is most active against *Staphylococcus aureus*. *Staphylococcus aureus* is a causal agent of various human infections such as bacteremia, infective endocarditis, osteoarticular, pleuropulmonary, skin and soft tissue infections (Tong et *al*., 2015). In addition, several studies have reported the effective and remarkable antibacterial activity of different fruit extracts of *Chamaerops humilis* L.; the activity includes both Gram positive and Gram negative microorganisms (Auwal et al., 2013; Mohamed et al., 2010). Similar anterior studies support the obtained results: the hexanic extract of the studied plant fruit revealed a substantial antibacterial activity against intestinal microflora and potential pathogens related to constipation, while the aqueous extract showed a moderate activity (Ewansiha et al., 2021). In the conducted investigation, it appears that Escherichia coli (Gram-) is the most resistant bacterium to all extracts tested to other strains (Gram+). This is due to the different wall structures of the bacteria. In effect, the cell walls of Gram+ bacteria are composed of a single layer, while those of Gram- bacteria have a multilayer structure, bound by an external cell membrane (Shtayeh et al., 1998). This renders the Gram- bacteria more resistant. It is well known that the efficiency of the antimicrobial activity depends on the nature of the phytochemicals that constitute each plant; therefore, their antimicrobial power can be varied from one plant to another against one organism to another (Mobina & Sudip, 2021).

CONCLUSIONS

This study presented a significant quantity of data concerning the leaves, fruits and pulp of *C. humilis* These three parts have distinct chemical and biological properties. The highest levels of polyphenols and flavonoids suggest that the leaves, fruits, and pulp may have applications in pharmacological and nutritional sciences. The highest levels of antioxidant activity and the highest efficiency of antibacterial activity of leaves, fruits and pulp offer good antioxidant and microorganism safety. Therefore, the results of this investigation should stimulate the interest of this plant for use in the pharmaceutical sector, food and other industries.

REFERENCES

1. Aazza S., B. Lyoussi, M. G. Miguel 2011. Antioxidant and Antiacetylcholinesterase Activities of Some Commercial Essential Oils and Their Major Compounds. Molecules. 16(9): 7672–7690.
2. Abdel-Hameed E.-S.S., Nagaty M.A., Salman M.S., Bazaidm S.A. 2014. Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (Opuntia ficus indica Mill.) growing in Taif, KSA. Food Chemistry. 160: 31–38.
3. Acherrat K. 2016. Activité antibactérienne des extraits flavonoïdiques de Chamaerops humilis L. Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers. Université de Tlemcen.
4. Al Akeel, Al-Sheikh Y., Mateen A., Syed R., Janardhan K., Gupta V.C. 2014. Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains. Saudi Journal Biological Sciences. 21(2): 147–151.
5. Alam Md.N., N.J.Bristi, Md.Rafiquzzaman 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi pharmaceutical journal. 21(2), 143–152.
6. Ali Shtayeh M.S., Yaghmour R.M.R., Faidi Y.R., et al. 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. Journal of Ethnopharmacol. 60(3): 265–271.
7. Amal A.M., Ashraf A. Khalil, Hossam E.S. El-Beltagi. 2010. Antioxidant and antimicrobial properties of kaff maryam(Anastatica hierochuntica) and doum palm (Hyphaene thebaica). Grasas y Aceites . 61(1), 67–75.
8. Aruoma O.I. 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation Research . 523–524: 9–20.
9. Auwal M.S., Mairiga I.A., Shuaibu A., et al 2013. Preliminary phytochemical and in vitro antibacterial evaluation of the crude pericarp extract of Hyphaene thebaica (doum palm). Journal of medicinal plant and herbal Therapy Research. 1(1): 1–7.
10. Belhaoues S. 2018. Étude phytochimique et activités biologiques des extraits de feuilles et de fruits de Chamaerops humilis L (PhD Thesis). 1–159.
11. Bengag A. 2009. Caractérisation phytochimique et activité antioxydante de quelques cultivars de Phoenix dectylifera L. Thèse de magistère. université d'Oran. 1–93.
12. Benmehdi H., Hasnaoui O., Benali O., Salhi F. 2011. Phytochemical investigation of leaves and fruits extracts of Chamaerops humilis L. Journal of Materials and Environmental Science. 3(2): 320–237
13. Berche P., Gaillard J.L., Simonet M. 1991. Les bactéries des infections humaines, Éd. Sciences," Flammarion Médecine. 660.
14. Billing J., Sherman P.V. 1998. Antimicrobial Function of Spice: Why Some Like it Hot. Review. Quarterly Review of Biology. 73: 3–49.

15. Blumenthal M., Busse W., Goldberg A., Gruenwald J., Hall T., Riggins C.W, Rister, R.S. 1998. The Complete German Commis-sion E Monographs: Therapeutic Guide to Herbal Medicines. American Botanical Council: Austin, TX. 136–138.
16. Bnouham M., Merhfour F.Z., Ziyyat A., Aziz M., Legssyer A., Mekhfi H. 2010. Antidiabetic effect of some medicinal plants of Oriental Morocco in neonatal non-insulin-dependent diabetes mellitus rats. Human & Experimental Toxicology. 29(10).
17. Bouhafsoun A., Ahmed B., Ismail Y., Lamine D. M., Tiguirangué D., Kheira E., Meriem K. H. 2019. Chemical composition and mineral contents of leaflets, rachis and fruits of Chamaerops humilis L. Annals: Food Science & Technology, 20(1).
18. Bouterfas K., Mehdadi Z., Elaoufi M.M., Latreche A., Benchiha W. 2016. Antioxidant activity and total phenolic and flavonoids content variations of leaves extracts of white Horehound (Marrubium vulgare Linné) from three geographical origins. Annales Pharmaceutiques Françaises. 74(6), 453–462.
19. Chaturvedi A., Singh S., Nag T.N. 2010. Antimicrobial activity of flavonoids from in vitro tissue culture and seeds of Gossypium species. Romanian Biotechnological Letters. 15(1): 1–5.
20. Chua L.S., Rahaman N.L.A., Adnan N.A., Eddie Tan T.T. 2013. Antioxidant activity of three honey samples in relation with their biochemical components. Journal of Analytical Methods in Chemistry. 8(1): 1–8.
21. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 2012. CLSI Document M07A9, Clinical and Laboratory Standards Institute. Wayne, PA, USA. (32). 1–34.
22. Coelho J.P., Veiga J.G., Elvas-Leitao R., Brigas A.F., Dias A.M., Oliveira M.C. 2017. Composition and in vitro antioxidants activity of Chamaerops humilis L. 1–5.
23. Cook J., Vander Jagt D., Dasgupta A., Glew R., Blackwell W., Glew R. 1998. Use of the Trolox assay to estimate the antioxidant content of seventeen edible wild plants of Niger. Life Sciences. 63(2) : 105–110.
24. Cowan M.M 1999. Plant products as antimicrobial agents. Clinical microbial agents. 12(4) : 564 –582.
25. Dimitrijevic D., Stankovi´c S.M., Stojanovic´-Radic Z., Ran\ d jelovi´c V., Laku˘ si´c D. 2012. Antioxidant and antimicrobial activity of different extracts from leaves and roots of Jovibarba heuffelii (Schott.) A. Löve and D. Löve. Journal of Medicinal Plants Research. 6(33). 4804–4810.
26. Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P., Vidal N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry. 97(4), 654–660.
27. El Cadi Hafssa, H. El Bouzidi, G. Selama, B. Ramdan, Y. Oulad El Majdoub, F. Alibrando, K. Arena, M. Palma Lovillo, J. Brigui, L. Mondello, F. Cacciola, Tania M.G. Salerno 2021. Elucidation of Antioxidant Compounds in Moroccan Chamaerops humilis L. Fruits by GC–MS and HPLC–MS Techniques. Molecules. 26(9): 1–13.
28. El-Beltagi H.S., Mohamed H.I., Yousef H.N., Fawzi E.M. 2018. Biological activities of the doum palm (Hyphaene thebaica L.) extract and its bioactive components. 1–1.9.
29. Eldahshan O., Ayoub N., Singab A., Al-Azizi M. 2009. Potential antioxidant phenolic metabolites from doum palm leaves. International journal of pharmacy and pharmacology. 2(10): 1–7.
30. Emad M., Abdallah, Adil A.H., Mujawah, Samiah H., Al-Mijalli 2021. Antibacterial Potential of Methanolic extract Hyphaene Thebaica L. Fruit Pulp against Antibiotics-resistant Pathogens. Journal of Pure Applied Microbiology . 15(3):1655–1664.
31. Ewansiha J.U., Ugo C.E., Kolawole D.I., Orji L.S. 2021. Antibacterial Activities of Hyphaene thebaica (Doum Palm) Fruit Extracts against Intestinal Microflora and Potential Constipation Associated Pathogens in Yola Metropolis, Nigeria. Tanzania Journal of Science. 47(1): 104–111.
32. Faten M.A.E. 2009. Antioxidant and anticancer activities of doum fruit extract (Hyphaene thebaica). African Journal of Pure and Applied Chemistry. 3(10). 197–201.
33. Forina M., Lanteri S., Armanino C. 1987. Chemometrics and Species Identification. Topics in Current Chemistry. Springer Berlin Heidelberg. 91–143.
34. Freitag H. 1971. Die naturiiche végétation des Sudotspanisenen Trockengebietes. [ouvrage].
35. Gonçalves S., Medronho J., Moreira E., Grosso C., Andrade P.B., Valentão P., Romano A. 2018. Bioactive properties of Chamaerops humilis L.: antioxidant and enzyme inhibiting activities of extracts from leaves, seeds, pulp and peel. 3 Biotech. 8(2), 88.
36. Goodman M., Bostick R.M., Kucuk O., Jones D.P. 2011 Clinical trials of antioxidants as cancer prevention agents: Past, present, and future. Free Radical Biology Medicine. 51(5):1068–1084.
37. Hagerman A.E., Riedal K.M, Jones G.A, Sovik K.N, Hartzfeld P.W, & Riechel T.L 1998. High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. Journal of Agricultural and Food Chemistry. 46(5):1887–1892.
38. Hasnaoui O., Adli D.E.H, Halla N. , Kahloula K. 2014 .Evaluation de L'activité Antifongique des huiles essentielles de Chamaerops humilis L. sur des souches isolées des Silos de stockage. Phytochem & biosub journal. 8(4): 222–228.
39. Hebi M., Eddouks M. 2016. Évaluation de l'activité antioxydante de Stevia rebaudiana. Phytothérapie. 14(1). 17–22.
40. Hindi N.K. 2013. In vitro antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar-garlic extract combination. American Journal of Phytomedicine and Clinical Therapeutics, 1(1): 42–51.

41. Hsu B., Coupar I.M., Ng K. 2006. Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. Food Chemistry. 98(2): 317–328.
42. Khoudali S., Essaqui A., Zertoubi M., Azzi M., Benaissa M. 2014. Study of antioxidant activity and anticorrosion action of the methanol extract of dwarf palm leaves (chamaerops humilis l.) from morocco. Journal of Materials ans environmental sciences. 5(3): 887–898.
43. Lantto T., Dorman D., Shikov B., Pozharitskaya O., Makarov V., Tikhonov P., Hiltunen P., Raasmaja A. 2009. Chemical composition, antioxidative activity and cell viability effects of a Siberian pine (Pinus sibirica Du Tour) extract. Food Chemistry. 112(4): 936–943.
44. Martins S., Amorim E.L.C, Sobrinho T.J.S.P, Saraiva A.M, Pisciottano M.N.C, Aguilar C.N, Mussatto S.I. 2013. Antibacterial activity of crude methanolic extract and fractions obtained from Larrea tridentata leaves. Industrial Crops and Products. Industrial Crops and Products. 41. 306–311.
45. Medjati N. 2014. Contribution à l'étude biologique et phytoécologique du Chamaerops humilis L. dans la partie occidentale de l'Algérie. Ph.D. Thesis, University of Tlemcen. 6(9): 143.
46. Miguel M.G., Nunes S., Dandlen S.A, Cavaco A.M, Antunes M.D 2010. Phenols and antioxidant activity of hydro-alcoholic extracts of propolis from Algarve, South of Portugal. Food Science and Technology 48(12):3418–23.
47. Mobina Parveen, Sudip Some. 2021. Antimicrobial Activity of Medicinal Plants: A Weapon to Combat Multi-Drug Resistant Pathogens. International Journal of Plant and Environment. 7(2): 133–14248.
48. Mohamed A.A., Khalil A.A., El-Beltagi H.E.S. 2010. Antioxidant and antimicrobial properties of kaff Maryam (Anastatica hierochuntica) and doum palm (Hyphaene thebaica). Grasas y Aceites. 61(1): 67–75.
49. Nile S.H., Nile A.S., Keum Y-S (2017). Total phenolics, antioxidant, antitumor, and enzyme inhibitory activity of Indian medicinal and aromatic plants extracted with different extraction methods. 3 Biotech. 7(1): 1–10.
50. Oladeji O. 2016. The Characteristics and Roles of Medicinal Plants: Some Important Medicinal Plants in Nigeria. Natural Products: An Indian Journal . 12(3): 1–8.
51. Olivier M.T., Muganza F.M., Shai L.J., Gololo S.S., Nemutavhanani L.D. 2017. Phytochemical screening, antioxidant and antibacterial activities of ethanol extracts of Asparagus suaveolens aerial parts. South African Journal of Botany. 108: 41–46.
52. Pisoschi A.M., G.P. Negulescu. 2011 "Methods for total antioxidant activity determination: a review. Biochemistry and Analytical Biochemistry 1(1): 3–12.
53. Prieto P., Pineda, M. 1999. Aguilar Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Analytical Biochemistry. 269(2): 337–341
54. Rice-Evans C., Miller N., Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine. 20(7): 933–956
55. Robards K., Prenzler P.D., Tucker G., Swatsitang P., Glover W. 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry. 66(4): 401–436
56. Seyfried H.E. 2007. Oxidative stress and antioxidants: a link to disease and prevention. Journal of Nutritional Biochemistry. 18(3): 168–171.
57. Simonne A.H., Kays S.J., Koehler P.E., Eitenmiller R.R. 1993. Assessment of β-Carotene Content in Sweetpotato Breeding Lines in Relation to Dietary Requirements. Journal of Food Composition and Analysis. 6(4): 336–345.
58. Singh S., Singh R.P. 2008. In Vitro Methods of Assay of Antioxidants: An Overview. 2008. Food Reviews International. 24(4) : 392–415
59. Suluvoy J.K., Grace V.M.B. 2017. Phytochemical profile and free radical nitric oxide (NO) scavenging activity of Averrhoa bilimbi L. fruit extract. 3 Biotech. 7(85): 1–12.
60. Surh Y.J, Chun K.S, Cha H.H, Han S.S, Keum Y.S, Park K.K, Lee S.S 2001. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutation Research, 480–481: 243–268.
61. Tabet N. 2006. Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine clothing. Age and Ageing. 35(4): 336–338
62. Taleb-Contini S.H., Salvador M.J., Watanabe E., Ito I.Y., Oliveira D.C.R.D 2003. Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. Brazilian Journal of Pharmaceutical Sciences. 39(4): 1–7
63. Tong S.Y., Davis J.S., Eichenberger E., Holland T.L., Fowler Jr. V.G. 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews. 28(3): 603–61.
64. Vernon L. Singleton, Rudolf Orthofer, Rosa M. Lamuela-Raventós 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 299: 152–178.
65. Wang B., Zhang W., Duan X., Li X. 2009. In vitro antioxidative activities of extract and semi-purified fractions of the marine red alga, Rhodomela confervoides (Rhodomelaceae). Food Chemistry. 113(4): 1101–1105.