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Antioxidant, antibacterial activities and phenolic content of organic fractions obtained from *Chamaerops humilis* L. leaf and fruit

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Key words: *Chamaerops humilis,* Antioxidant activity, Antibacterial activity, Polyphenols

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Abstract

The use of plant extracts are the subject of numerous studies because, in addition to their use as preservatives in foodstuffs by replacing synthetic antioxidants, they are involved in the treatment of many diseases. This study investigated the antioxidant and antibacterial activities of *Chamaerops humilis* L. (Asteraceae) leaf and fruit, collected in north east of Algeria and their respective fractions, obtained using dichloromethane (DCMF), ethyl acetate (EAF), n-butanol (BF) and water (WF). The total phenolic and total flavonoids content was determined by the Folin-Ciocalteu and the aluminum chloride (2%) reaction, respectively. Antioxidant activity was measured, using different antioxidant assays (DPPH, FRAP, BCB). The antibacterial activities of the fractions were tested on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis* and *Salmonella typhimurium*. Two different methods were employed for the determination of antibacterial activities: disc diffusion method and minimum inhibitory concentrations (MICs). A significant correlation existed between concentrations of the extract and antioxidant activity since the maximum antioxidative activities were exhibited by ethyl acetate fraction [IC_{50}=0.12 mg/ml (DPPH assay)] which has the highest levels of phenolic compounds (214,8±3,26 mg GAE/g fractions).Concerning antibacterial activity , the lowest activity was founded on the (WF)from fruits against *Salmonella typhimurium* (MIC=70mg/ml)and the highest was found on the (EA) fraction from leaves against *Enterococcus faecalis* (MIC=0,25 mg/ml) . These findings demonstrate the remarkable potential of this plant as new source of antioxidant ingredient with interesting antibacterial capacities.

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Introduction

Secondary metabolites such as polyphenols are chemical compounds, biosynthesized by plants during normal metabolic developments to prevent biotic and abiotic stresses they can undergo (Michel *et al.*, 2011; Olivier *et al.*, 2017). In the recent years, polyphenols have received much attention, since these bioactive substances exhibit a wide range of pharmacological properties, such as anti-allergic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective and vasodilatory effects (Matkowski *et al.*, 2008).

Many authors, in fact reported that the consumption of vegetables and fruits containing antioxidant phytochemicals (notably polyphenols) is advantageous for our health, as they can protect the human body from the disorders related to excessive oxidation of cellular substrates (oxidative stress) and retard the progress of many chronic diseases (Ordonez *et al.*, 2006; Lopes Lutz *et al.*, 2008).

On the other hand, the frequent use of antibiotics has increased the rapid emergence of drug-resistant germs (Bouacha *et al.*, 2015). For this reason there is a growing interest to the development and isolation for new therapeutic alternatives from plants.

Chamaerops humilis L. (Mediterranean dwarf palm) belonging to family Arecaceae, commonly named 'doum' is an important floristic element of the western Mediterranean region because it is the only palm species naturally distributed in both Europe and Africa (Guzman *et al.*, 2017). Leaves of this species are used in traditional medicine in Algeria and Morocco for the treatment of diabetes, digestive disorders, spasm, and gastrointestinal disorders (Benmehdi *et al.*, 2012). In addition, the fruit (bay) of this dwarf palm is alleged to have anti-inflammatory, urinary antiseptic and diuretic activities (Blumenthal *et al.*, 2000; Beghalia *et al.*, 2008). Additionally, several biologically important secondary metabolites such as Steroids Flavonoids, Phenols, Saponins, reducing compounds, Gallic tannins and Terpenoids have been detected from the leaves and fruits of *Chamaerops humilis* L. (Benmehdi *et al.*, 2012).

The purpose of the present study was to determine polyphenol, and flavonoid, contents at leaves and fruits as well as their antioxidant and antibacterial activities. Nevertheless, to the best of our knowledge, no studies about the antioxidant and antibacterial activities of specific fractions from *Chamaerops humilis* L. leaves and fruits have been reported.

Materials and methods

Plant material

Chamaerops humilis leaves and fruits were collected during autumn season (October, 2014), from the mountain ranges of Edough (Annaba, northeastern of Algeria). The plant was botanically identified by the department of botany (Annaba University). The leaves and fruits were washed thoroughly to remove dirt, then cut into small pieces and shade dried at room temperature for 15 days. Thereafter, dried plant materials were powdered and used for extraction.

Extraction and fractionation

The powders of leaves and fruits (100g) of *Chamerops humilis* were individually macerated in 80% methanol solution for 24h under stirring condition.

The raw extracts were filtered, and evaporated to dryness in a rotary vacuum evaporator (Buchi, Switzerland) at 40°C to obtain a crude methanol extract, and the residue was re-extracted twice following the same procedure. The dried methanol extract was dissolved in water and then successively exhausted with solvents of increasing polarity: dichloromethane (DCM), ethyl acetate (EA), and n-butanol (B).Water fraction (WF) corresponds to aqueous residue obtained at the end of the conventional liquid–liquid extraction (Michael *et al.*, 2012). The pooled fractions were evaporated to dryness; all the fractions were weighed, and stored at -18°C for further assays.

Determination of total phenolic content

Total phenolic content was determined by spectrometry using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Two hundred microliters of diluted sample were added to 1 ml of 1:10 diluted Folin-Ciocalteu reagent.

After 4 min, 800 µl of saturated sodium carbonate (75 g/l) was added. After stirring and 2 h of incubation at room temperature in the dark, the absorbance at 765 nm was measured. Gallic acid (0–200 µg/ml) was used for the standard calibration curve. The results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g fractions).

Estimation of flavonoids content

The flavonoids content in extracts was determined according procedures described by Arvouet-Grand *et al.* (1994), using a method based on the formation of a complex flavonoid–aluminum, having the maximum absorptivity at 430 nm. Briefly, 1 ml of diluted sample was mixed with an equal volume of aluminum trichloride ($AlCl_3$) in methanol. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with spectrophotometer. Quercetin (0-40 µg/ml) was used for the standard calibration curve, and results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g fractions).

Antioxidant activity

DPPH radical scavenging activity: The free radical scavenging activity of the sample was measured in vitro by DPPH (1, 1 diphenyl 2, picrylhydrazyl) (Sigma, Germany) assay, according to the method of Blois (1958) with some modifications. A methanolic solution (100µl) of the compound to be tested was prepared at different concentrations (100–1000µg/ ml) and was added to 1.90 ml of DPPH solution (6 $\times 10^{-5}$ M in methanol).

The mixture was vigorously shaken and incubated for 30 min in obscurity, and the absorbance was measured at 515 nm against corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading using the following equation:

DPPH Scavenged (%) = (A_{DPPH} – A_{sample})/A_{DPPH} × 100.

Where A_{DPPH} is the absorbance of the control reaction and A sample is the absorbance in the presence of the extract/standard.

The antioxidant activity of the extract was expressed as IC50, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. A low IC50 value represents a high antioxidant activity.

Determination of antioxidant activity with the beta carotene bleaching (BCB) test: Antioxidant activity of the plant extract was measured according to the procedures described by Pratt (1980). A solution of β-carotene was prepared by dissolving 0.5 mg of β-carotene in 1 ml of chloroform, 25µl of linoleic acid and 200 mg of Tween 40. After evaporation of the chloroform, under vacuum at 50°C by a rotary evaporator, oxygenated distilled water (100 ml) was added with vigorous shaking. Aliquots (2.5 ml) of this emulsion were transferred into different test tubes containing (350µl) of test samples extracts. The tubes were shaken and incubated at 50°C in a water bath. Butylhydroxytoluène (BHT) was used as positive control for comparative purposes. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 490 nm using a spectrophotometer against a blank, consisting of an emulsion without β-carotene. Absorbance readings were then recorded at 20 min intervals for 120 min. A mixture prepared, without sample, served as negative control. All determinations were performed in triplicate. The percentage inhibition was calculated using the following equation:

% inhibition = (β -carotene content after 2 h of assay/initial β -carotene content) × 100

Reducing power: The reducing power was determined according to the method of Oyaizu (1986). 1 ml of various concentrations of test samples extracts (0.1, 0.25, 0.5, 0.75, 1mg/ml) were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min.

After 2.5 ml of 10% trichloroacetic acid (TCA) were added, the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference. An increase in the absorbance of the reaction mixture indicated increased reducing and the extract concentration providing 0.5 of absorbance (IC50) was calculated from the graph of absorbance.

Antibacterial activity assays

Disc diffusion method: Antimicrobial activity was tested against a panel of bacterial standard strains. All these microorganisms were obtained from American Type Culture Collection (ATCC).The disc diffusion method (NCCLS, 2000a) was applied for the determination of antibacterial activities of the fractions from *Chamaerops humilis* leaves and fruits. Briefly, all the samples/fractions were dissolved in dimethyl sulfoxide (DMSO 5%, v/v) to obtain a concentration of 200 mg/ml. The bacterial cell suspension was prepared from 24 h culture and adjusted to an inoculation of 10^6 colony forming unit (CFU/ml) then was spread over the surface of agar plates Mueller Hinton agar (MHA) using sterile swabs into Petri dishes. Filter papers having a diameter of 6 mm, soaked with 100 µl samples (20mg per disk) and were placed on the inoculated agar plates. DMSO 5% was used as a negative control and antibiotic (*Imipenem* 15µg) was used as positive control for comparison. Before incubation, all Petri dishes were kept in the refrigerator (4°C) for 1 h to allow the complete diffusion of the samples. Then they were incubated at 37°C for 24 h. After incubation the diameters of inhibition zones were measured in mm.

Evaluation of minimum inhibitory concentration (MIC): The MIC (minimum inhibitory concentration) corresponds to the lowest concentration of tested compounds, able to inhibit any visible microbial growth (Muanda *et al.*, 2011). MIC evaluations were performed by the micro dilution assay according to the standard procedure of the Clinical and Laboratory Standards Institute (CLSI 2010), with some modifications.

Inoculum of bacteria strains were adjusted to approximately 10^6 CFU (colony forming unit) in Mueller–Hinton Broth (MHB), then 0.5 ml of inoculum was added to each tube containing 0.5 ml of compounds at various concentrations from 0.625 to 70 mg /ml in DMSO 5%.

All the tubes were incubated at 37°C for 24 h and growth or no-growth was assessed by the naked eye, then MIC was determined.

Statistical analysis

Statistical analysis of the data was performed under R software (Version 3.3.2, for Windows).Data were reported as mean (∓) standard deviation of 3 replicates. Analysis of variance (ANOVA) accompanied with Tukey test were conducted to identify the significant differences between the samples ($p<0.05$). Correlations between phenolic content, flavonoids content and antioxidant activity were examined using Pearson's test.

Results and discussion

Extraction and fractionation yield

Methanolic crude extract of each organ (leaf and fruit) were separated in four fractions by classic liquid–liquid extraction (LLE) using dichloromethane (DCMF), ethyl acetate (EAF), n-butanol (BF), and water (WF). The mass yields are given in Fig.1.

In general, the amount of total extractable compounds decreased with decreasing polarity of the solvent in the order of water, butanol, ethyl acetate and dichloromethane. For both organs water (WF), and butanol (BF) fractions were always exhibited the higher content (8, 53% and 6.26%, of dry leaves weight, respectively) and (10.67 % and 8.75%, of dry Fruits weight respectively). Concerning (DCMF) and (EAF) fractions, we record the lowest yields of 0.84% and 1.34%, of dry leaves weight, respectively and 3.41% and 1.12% of dry fruits weight, respectively. Our result is in agreement with other studies on other plants which have indicated a maximum average yield in aqueous fraction (Dhingra *et al.*, 2017).

Thus, the extraction yields vary according to the plant under study and the physicochemical characteristics of the solvents used, and in particular their polarity.

Total phenolic content

The Various fractions (from leaves and fruit) of *Chamaerops humilis* showed different levels of total polyphenols and total flavonoids, the concentrations obtained were summarized in Table 1.

Table 1. Total phenolic and flavonoid contents of different fractions of *Chamaerops humilis*.

Organs	Test fractions	Total phenolic contents (mg GAE/g fractions)	Total flavonoid content (mg Quercetin E/g fractions)
Leaf	DCMF	56,53±4,82^{c}	54,27±0,94^{a}
	EAF	214,80±3,26^{a}	45,16±0,43^{b}
	BF	106,75±4,55^{b}	24,71±1,04^{c}
	WF	24,29±0,30^{e}	2,36±0,19^{e}
Fruit	DCMF	12,71±1,20^{f}	3,23±0,10^{e}
	EAF	37,86±0,16^{d}	4,85±0,20^{d}
	BF	9,41±0,07^{f}	1,71±0,11^{e}
	WF	5,76±0,17^{f}	1,19±0,02^{e}

Values followed by different letters in a column are significantly different (P < 0.05) according to ANOVA and Tukey test. DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolicfraction; WF, water fraction.

Phenolic contents were expressed as milligrams of gallic acid equivalents per gram of dry matter. The results indicate that the total polyphenols contents vary significantly according to the solvent used for fractionation, with grades ranging from 24.29 mg to 214.8mg equivalent gallic acid/g dry matter for leaves' fractions and from 5.76 mg to 37.86 mg equivalent gallic acid/g dry matter for fruits' fractions. Thus, in both organs studied acetate ethyl fractions (EAF) had the highest amount of phenolic followed by butanolic fractions (BF), and dichloromethane fractions (DCMF). Water fractions (WF) had the lowest phenolic contents. In addition, the phenolic contents were significantly higher in leaves ($p < 0.05$) than fruits. These findings agree with previous reports (Benhammou *et al.*, 2009) indicating that secondary metabolites distribution may fluctuate between different plant organs.

Table 2. IC50 values (mg/ml) and percentage of inhibition of *Chamaerops humilis* extracts in the antioxidant activity evaluation assays.

Organs	Test fractions /standard	DPPH (IC_{50} mg/ml [a])	BCB (Percentage of inhibition %)	FRAP (IC_{50} mg/ml [b])
Leaf	DCMF	0,81^{b}	57,66^{b}	1,06^{d}
	EAF	0,12^{a}	66,24^{a}	0,19^{b}
	BF	0,53^{b}	68,51^{a}	0,62^{c}
	WF	1,37^{c}	56,69^{b}	0,90^{d}
Fruit	DCMF	2,67^{d}	52,05^{c}	1,60^{f}
	EAF	0,76^{b}	60,06^{b}	1,22^{e}
	BF	4,95^{f}	52,11^{c}	10,18^{h}
	WF	3,73^{e}	48,88^{c}	4,43^{g}
	Ascorbic acid	0,07^{a}	-	0,06^{a}
	BHT	-	72,38^{a}	-

[a]IC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged. [b] IC_{50} (mg/ml): effective concentration at which the absorbance is 0,5.

Values followed by different letters in a column are significantly different (P < 0.05) according to ANOVA and Tukey test. DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolic fraction; WF, water fraction.

Total flavonoid content

Flavonoids are the main group of phenolic compounds. Most Studies has shown that plant extracts rich in flavonoids are a good source of antioxidants (Djeridane *et al.*, 2006; Bouterfas *et al.*, 2016). The total flavonoid content was analyzed. Results are presented in Table 1 and they indicate that highest amount of total flavonoids content was found in DCM fraction (54.27 ± 0,94 mg equivalent quercetin/g), while minimum was present in WF (2.36 ± 0.19 mg equivalent quercetin/g) in case of leaves. Concerning fruit, all fractions exhibit poor contents of flavonoids ranging from (1.19 ± 0.02 to 4.85 ± 0.2 mg equivalent quercetin/g).

Table 3. Antibacterial activity of fractions from leaf and fruit tested at 20mg/ml, and expressed as growth inhibition zone (mm).

Organs	Sample [a]	Tested micro-organisms[b] /inhibition zone (mm)[c].					
		Ec	Pa	St	Kp	Sa	Ef
Leaf	DCMF	-	8,33 ±0,57f	10±00 [b]	11,33±0,23 [b]	-	-
	EAF	13,33±0,57[c]	18,33±0,66[c]	15±10[a]	20±10[a]	14,33±0,5[a]	16,33±0,57 [c]
	BF	11,33±0,33[e]	11,66±0,57[e]	-	13,33±0,57[b]	12,66±0,5[a]	15±10[c]
	WF	11,66±1,15[c]	13,66±0,57[d]	11,66±0,57[b]	12,33±0,57 [b]	11±10 [b]	13±10[d]
Fruit	DCMF	7,66±0,57[d]	7,33±0,57 [f]	7±0,57[c]	-	9,33±1,52 [b]	13±00[d]
	EAF	17,33±1,15[b]	21±10 [b]	11,33±0,57[b]	10,66±00 [b]	14,33±0,5[a]	19,33±0,57[b]
	BF	10,33±1,52[e]	7,33±0,57 [f]	12,66±00[b]	-	9,33±1,15[b]	9,66±0,57[e]
	WF	-	-	-	-	-	-
	Imipenem	29,66±0,66[a]	41,33±10 [a]	-	-	-	41,33±0,3[a]

(–) no growth inhibition zone observed. Values followed by different letters in a column are significantly different ($P < 0.05$) according to ANOVA and Tukey tests.

[a]The tested samples were DCMF: dichloromethane fraction; EAF: ethyl acetate fraction; BF: butanolic fraction; WF: water fraction; Imipenem: antibiotic

[b]Tested microorganisms: Ec: *Escherichia coli; Pa: Pseudomonas aeruginosa; St: Salmonella typhimurium; Kp: Klebsiella pneumonia; Sa: Staphylococcus aureus; Ef: Enterococcus faecalis.*

[c]Including the diameter of the disk (6 mm).

Compared to other extracts of plants reported in literature, the extract obtained, in this study, from *Chamaerops humilis* (Especially for leaves) was more rich in polyphenols and flavonoids than those in some Asian plants such as *Prunus dulcis* (Dhingra *et al.*, 2017) and *Nigella sativa* (Mariod *et al.*, 2009). Indeed, Djeridane *et al.* (2007) who evaluated several Algerian medicinal plants found that this abundance is characteristic of the Asteraceae family in North Africa. The authors suggested that those characteristics may be related to the hard climate conditions of Asteraceae usual habitat (hot temperature, high solar exposure, drought, salinity), which stimulate the biosynthesis of secondary metabolites such as polyphenols.

In addition, the EA fraction exhibited the highest polyphenols contents as reported by other authors who studied North African plants (Salem *et al.*, 2011; Khadraoui *et al.*, 2015).

Antioxidant activity

To investigate the antioxidant activities of *Chamaerops humilis* organs we used three different biochemical in vitro assays: the DPPH radical scavenging, the reducing power, and the inhibition by β-carotene-linoleate system (BCB), which are based on different mechanisms of action.

DPPH: DPPH has been widely used in the determination of the antioxidant activity because it is fast, easy and reliable and does not require a special reaction and device (Aksoy *et al.*, 2013).

The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. The radical scavenging activity of fractions (DCMF, EAF, BF, WF) of leaves and fruits of *Chamaerops humilis* are expressed in terms of IC50, and shown in Table 2.

The IC 50 of all fractions from leaves and fruits ranged from 0.12mg/ml to 3.7mg/ml and 0.76mg/ml to 4.95mg/ml, respectively. EA fractions showed the highest activity in both leaves and fruits, 0.12 mg/ml and 0.76 mg/ml, respectively, However, DPPH free radical scavenging of all fractions tested was significantly less (p<0,05) than that of ascorbic acid, a synthetic antioxidant (0.07 mg/ml) except ethyl acetate leaves' fraction (p>0,05). Furthermore leaves showed lower IC50 value than fruits (p<0, 05). These findings may be due to the presence of higher amount of total phenolic and flavonoids in leaves as compared to fruits. These results are in accordance with those of many studies which reported a correlation between the polyphenols and flavonoids contents of extracts and radical scavenging activity; they also observed a higher activity for the ethyl acetate fraction (Benhamou *et al.*, 2009; Salem *et al.*, 2011).

Table 4. Minimum inhibitory concentration (MIC, in mg/ml) of fractions obtained from *Chamaerops humilis* leaves and fruits on different bacterial strains.

Organs	Sample[a]	Tested microorganisms[b]/ MIC(mg/ml)					
		Ec	Pa	St	Kp	Sa	Ef
Leaf	DCMF	2	4	20	10	20	2
	EAF	2	0,5	4	2	2	0,25
	BF	20	10	10	10	10	4
	WF	40	40	50	60	40	50
Fruit	DCMF	10	4	10	4	4	4
	EAF	4	2	0,5	1	2	1
	BF	10	10	4	4	4	4
	WF	50	50	70	40	40	50

[a]The tested samples were DCMF: dichloromethane fraction; EAF: ethyl acetate fraction; BF: butanolic fraction; WF: water fraction; Imipenem: antibiotic

[b]Tested microorganisms: Ec: *Escherichia coli; Pa: Pseudomonas aeruginosa; St: Salmonella typhimurium; Kp: Klebsiella pneumonia; Sa: Staphylococcus aureus; Ef: Enterococcus faecalis.*

Benhamou *et al.* (2009) showed that the acetate ethyl fraction of *Atriplex halimus* (Amaranthaceae family), exhibited a stronger antioxidant activity compared to butanolic fraction with IC50 values 1.73mg/ml (R^2 = 0.98) and 2.04 (R^2 = 0.99) mg/ml, respectively. Also, Dhingra *et al.* (2017) observed a significant positive correlation between the total phenolic contents of *Prunus dulcis* and DPPH scavenging with R^2 = 0.931.In this study we found a significant correlation between the antioxidant activity and phenolic content with coefficient of determination (R^2> 0, 91, p<0, 05) for all samples tested.

Beta carotene bleaching (BCB) test: The BCB method is based on the loss of the orange color of β -carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. However, the presence of antioxidant constituents could neutralize the linoleate free radical and hence prevent the bleaching of β-carotene (Chew *et al.*, 2008).

The results obtained in this assay indicated that all fractions of both organs studied (leaves and fruits) had a higher antioxidant capacity and a higher activity compared with the negative control (P<0, 05) (without addition of extract) Fig. 2 and 3.

However, except ethyl acetate and butanolic fraction from leaves, all compounds showed a significantly lower ($p<0, 05$) activity than that of BHT, a synthetic antioxidant (positive control) (Table2). The results revealed also that the antioxidant activity of fractions from leaves had better antioxidant properties than those of fruits. This finding is in agreement with the DPPH assay. Thus, the comparison of the degradation rate of β-carotene between the fractions allowed us to establish this order:

BHT (72,38%)> BF (68,51%)> EAF (66,24%)> DCMF (57,66%)> WF (56,69%) for leaves and BHT (72,38%)> EAF (60,06%)> BF (52,11%)> DCMF (52,05%)> WF (48,88%) for fruits. Overall, EAF and BF fractions revealed better antioxidant properties than did either DCMF or WF. These results are in agreement with the high content of polyphenols and flavonoids found in these fractions. Similar results were found on others plants by Chan *et al.* (2014) authors who found a positive correlation between the capacity to inhibit bleaching of β-carotene and the high amount of polyphenols.

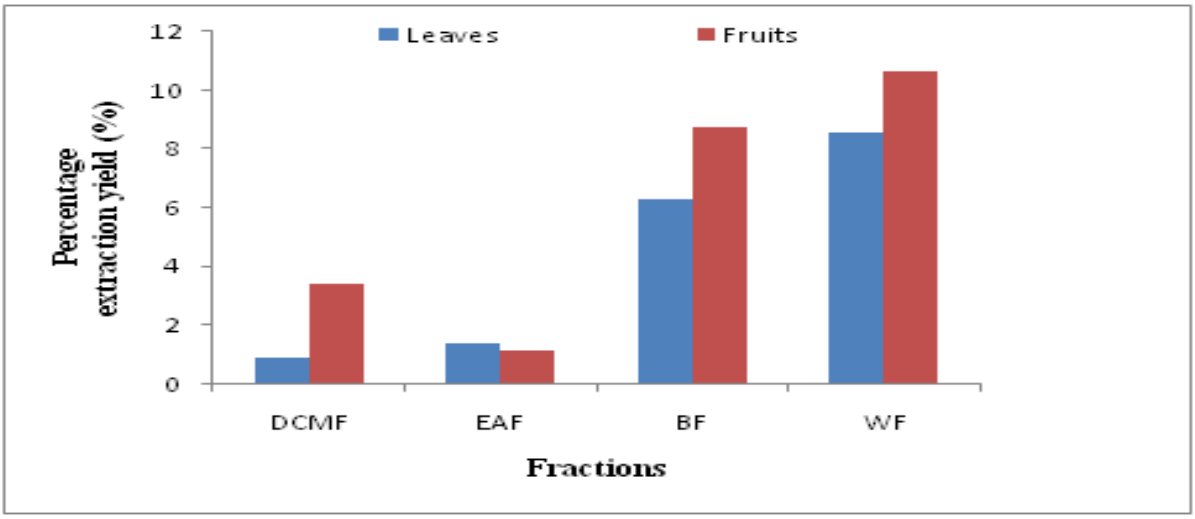

Fig. 1. Mass yields of fractions obtained from the methanolic extract of *Chamaerops humilis* leaf, and fruit. DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolic fraction; WF, water fraction.

Frap assay: The reducing power assay consists of evaluating the ability of plant fractions to reduce Fe^{3+} ions to Fe^{2+} by electron donation (Olivier *et al.*, 2017). The absorbance obtained for all the extracts and ascorbic acid are shown in Fig.4 and5.And their IC50 values are represented in Table 2.

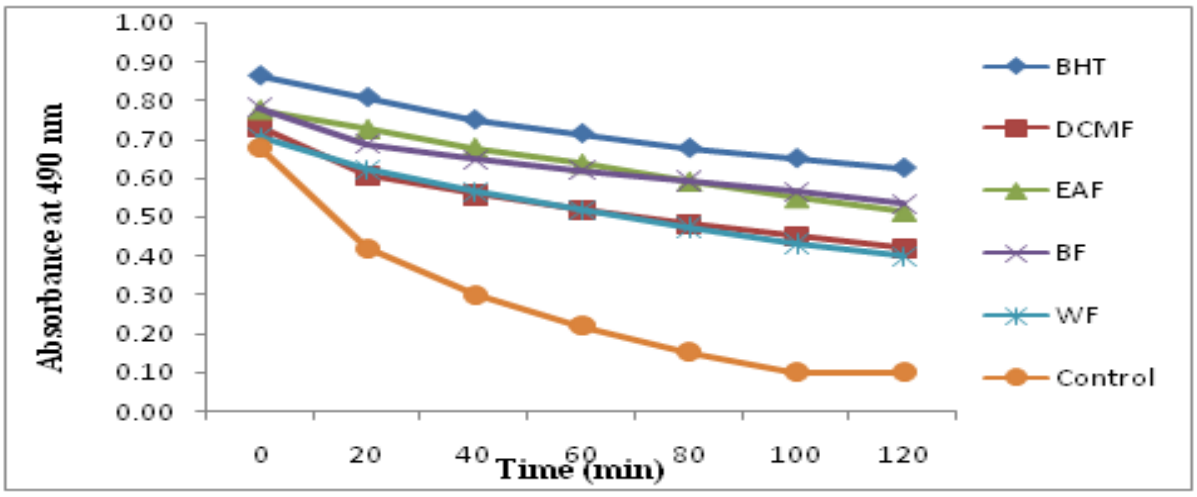

Fig. 2. Effect of *Chamaerops humilis* leaves 'fractions and BHT (Butylhydroxytoluène) standard on oxidation of β-carotene/linoleic acid.

DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolic fraction; WF, water fraction.

The IC 50 of all fractions from leaves and fruits ranged from 0.19 mg/ml to 0.9 mg/ml and 1.22mg/ml to 10.18mg/ml, respectively. EA fractions showed the highest activity. However, this effect was not as great as the effect of ascorbic acid ($p<0.05$).

In this assay, we noted the correlation coefficient between phenolics (total polyphenol and flavonoids) and values of the reducing power activity was highly significant ($R^2> 0, 86$) for all compounds, indicating that polyphenols may play an important role in the reducing power. Oliver *et al.* (2017) reported the IC 50 for hexane, chloroform and butanolic fractions of *Asparagus suaveolens* at 2.5mg, 2.5mg and 1.87 mg respectively. Our extract (especially leaves' fractions) demonstrated a stronger reducing power.

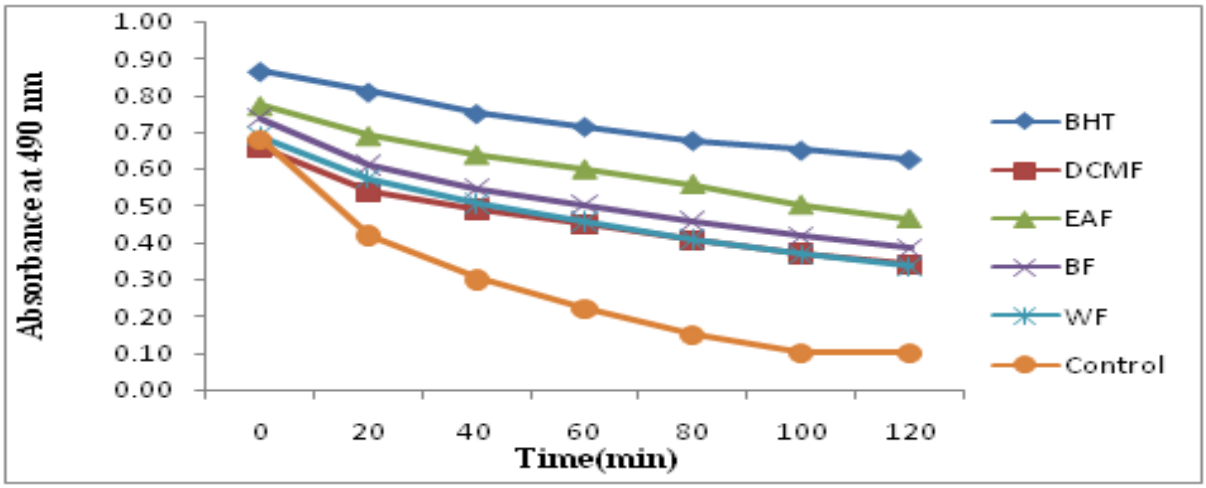

Fig. 3. Effect of *Chamaerops humilis* fruits 'fractions and BHT (Butylhydroxytoluène) standard on oxidation of β-carotene/linoleic acid.

DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolic fraction; WF, water fraction.

Butanolic and water fractions from fruits were less active than the other fractions as well as the standards (ascorbic acid). This suggests that these fractions are not electron donors, and cannot reduce Fe^{3+} ions to Fe^{2+}. Therefore, the antioxidant activity is not only dependent on the concentration, but also on the structure and the nature of the antioxidants (Falleh *et al.*, 2008). Based on these results, it may be concluded that the antioxidant effect of polyphenols and flavonoids is not only dose dependent but could also be structure-dependent. Indeed, many authors established that the antioxidant activity is positively correlated with polyphenols structure (Li *et al.*, 2012; Bouterfas *et al.*, 2016).

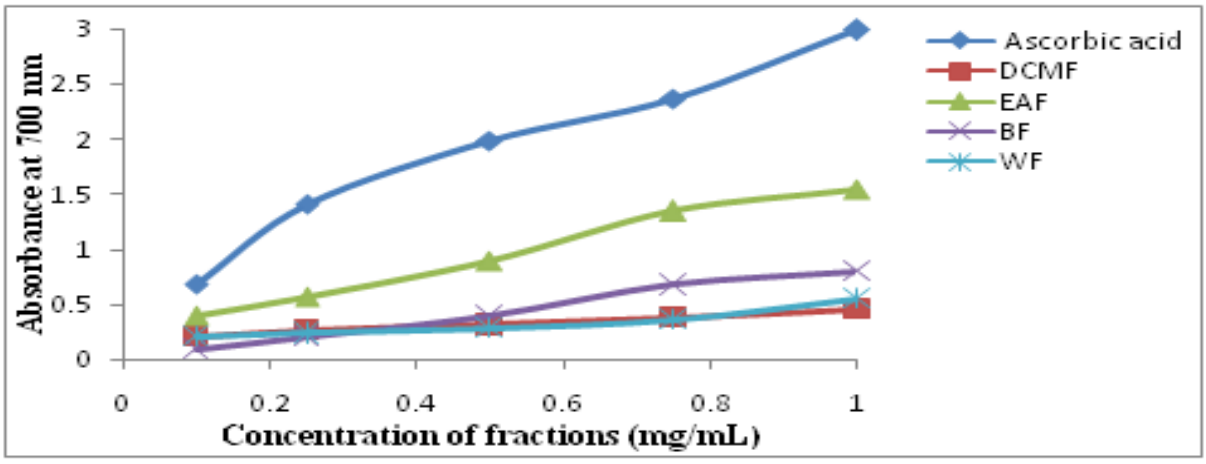

Fig. 4. Reducing power of *Chamaerops humilis* leaves 'fractions.

DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolic fraction; WF, water fraction

Antibacterial activities

Disc diffusion method: The agar disc diffusion technique is frequently used to screen plant extracts for antimicrobial activity (Lopes-Lutz *et al.*, 2008). As can be seen the antibacterial activity of *Chamaerops humilis* extracts was found against both some Gram + and Gram – bacterial species with varying degrees of inhibition (p<0, 05) (Table 3).

All the samples from leaves and fruits presented an inhibitory activity except the WF from fruits. For the fractions from leaves, it is evident that the ethyl acetate fraction exhibits maximum antibacterial potency against all the bacteria tested. The maximum zone of inhibition of EA fraction was 20 mm and 18mm recorded against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. *Escherichia coli* were found to be less sensitive with 13mm zone of inhibition. Butanolic and water fractions displayed moderate activity, while the DCM fraction showed very little zone of inhibition compared to the other fractions. The results indicated also that DMSO 5%, used as negative control, did not produce any inhibition zone on its own. In contrast, *Imipenem*, used as positive control produced, 29mm, 41mm, and 43mm inhibition zones with *Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively, and it did not show any activity on the other bacterial strains.

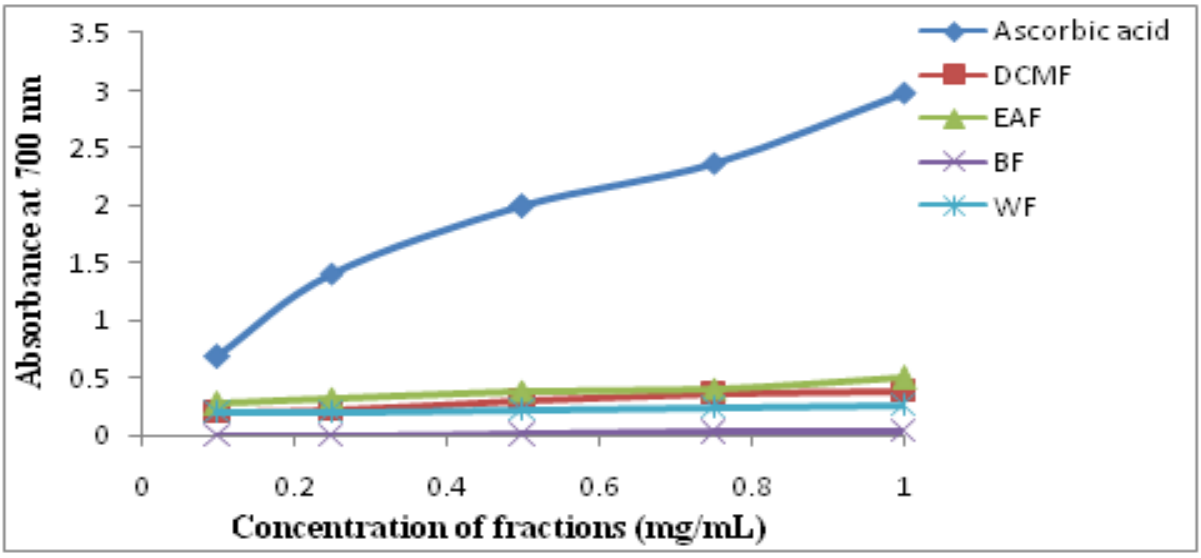

Fig. 5. Reducing power of *Chamerops humilis* fruits 'fractions DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; BF, butanolic fraction; WF, water fraction.

In general, the Gram-negative bacteria showed less sensitivity to plant extract. This may be due to their extra lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial (Tiwari *et al.*, 2014). However, this result was not observed in our study since the extracts had an effect almost similar and sometimes even higher upon gram - than gram+. These results suggest that the presence of phytochemicals have a broad spectrum antibacterial activity.

Regarding to the antibacterial activity of fractions from fruits, the most interesting results were found, for the EA fraction. Indeed this fraction was the most efficient, particularly for *Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis* recording a maximum of 17mm, 21mm, and 19 mm zone of inhibition, respectively. Furthermore, this fraction showed the most relevant antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis* compared to the ethyl acetate fraction from leaves which has the highest levels of phenolic compounds (Table 1).

This suggests that antibacterial activity of EA fraction from fruits may be attributed to other classes of secondary metabolites.

In fact, according to many reports (Al Akeel *et al*., 2014; Sharifirad *et al*., 2014; Olivier *et al*., 2017) the presence of alkaloids, terpenoids, glycosides steroids and proteins may be responsible for the antibacterial properties of plant extracts. Moreover, other authors (Lopes-Lutz *et al*., 2008; Martins *et al*., 2013) reported also, the difficulty to attribute the activity of a complex mixture to a single constituent and that the efficiency of plants extracts might be explained by synergistic or additive effects of several phytochemicals.

Minimum inhibitory concentration (MIC): The real extend of antibacterial activity is evaluated by determining MIC values (Martins *et al*., 2013). The results of MIC against bacterial strains are summarized in Table 4.

Both ethyl acetate from leaves and fruits showed the highest activity and were found to be in the range of 0.25 to 4mg/ml, and from 0.5 to 4mg/ml respectively, while DCMF and BF fractions showed a moderate activity. On the other hand, WF exhibited very low antibacterial activities with MIC > 40 mg for all the tested bacteria. Overall, the results obtained in these assays revealed that *Pseudomonas aeruginosa* and *Enterococcus faecalis* were the most sensitive bacteria to EA fraction from leaves with a MIC value of 0.5mg/ml and 0.25mg/ml, respectively, while *Salmonella typhimurium* exhibited the highest resistance (MIC=4mg/ml). Enterococcus *faecalis, Salmonella typhimurium* and *Klebsiella pneumonia* showed a better sensitivity against EA fraction from fruits with a MIC value of 1mg/ml, 1mg/ml, and 0.5mg/ml respectively, while *Escherichia coli* exhibited the highest resistance (MIC=4mg/ml). No relevant results were obtained for the others fractions.

As can be seen, different performances of fractions tested were observed between agar diffusion and MIC assays, especially for DCM fraction which exhibited a moderate activity with MIC and a weak activity with agar diffusion. This difference could be attributed to the polarity.

Hammer *et al.* (1999) reported that agar disc diffusion was a limited technique as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium. In fact, DCMF is an apolar fraction, and contents a hydrophobic compound like aglycone and long carbon chain ones which prevents the good diffusion of this extract in the agar. Concerning our results it is obvious that antibacterial activity of DCMF is influenced by the polarity of agar.

As previously reported (Ravikumar *et al*., 2007; Martins *et al*., 2013), the EA fractions exhibited potential activity against the reference strains of bacteria. This finding is in agreement with our study. However, comparing our results to those obtained with other plants in the literature is problematic since the choice of test microorganisms and the method used to assess antimicrobial activity varies between publications (Hammer *et al.* 1999).

Conlusion

The results of the present study demonstrated that phytochemicals from *Chamaerops humilis* leaves and fruits had antioxidant and antibacterial properties. Thus, these compounds can be a potential resource of natural antioxidants to be used in functional foods and health, and a treatment of infectious diseases caused by some pathogenic bacteria. Furthermore, the ethyl acetate fraction was found to possess better antioxidant and antibacterial potentials among the entire fraction evaluated.

However, further investigations are required to know the active substance responsible for the antioxidant and antibacterial potency of *Chamaerops humilis* and its mode of action.

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