Journal of Applied and Natural Science
11(1): 144-148 (2019)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Determination of anticholinesterase and antioxidant activities of methanol and water extracts of leaves and fruits of *Chamaerops humilis* L.

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Article Info
DOI:10.31018/jans.v11i1.2001
Received: January 26, 2019
Revised: February 17, 2019
Accepted: February 20, 2019

How to Cite
Bouhafsoun, A. *et al.* (2019). Determination of anticholinesterase and antioxidant activities of methanol and water extracts of leaves and fruits of *Chamaerops humilis* L.. *Journal of Applied and Natural Science*, 11(1): 144-148

Abstract
The present study was carried out to investigate the antioxidant activity of the water and methanol extracts of leaves and fruits extracts of *Chamaerops humilis* L. by using ABTS cation radicals and cupric reducing antioxidant capacity (CUPRAC). Anticholinesterase effect of the extracts was tested against both AChE and BChE using a microplate-reader assay based on the Ellman method. The methanol extracts of *C. humilis* leaves contained relatively higher content of flavonoids and total phenolics than those of fruits. All the extracts were found to have different levels of antioxidant activity in the systems tested. The leave extract showed the highest value of antioxidant activity, based on ABTS radical-scavenging activity, while the fruit water extract showed the highest value (0.53±0.50 µg/mL) of cupric reducing antioxidant activity. Our data indicates that both methanol and water fruit extract were active for BChE inhibition (31.65 ± 0.37 and 30.19 ± 0.56%) respectively, whereas, all leave extracts did not show any activity against BChE. The present study demonstrated that the methanol and water extracts fractions of *C. humilis* have different responses with different antioxidant methods. Our results suggest that the *C. humilis* could be used as a source of antioxidant agents and may be beneficial in the AD treatment.

Keywords: Biological activity, *Chamaerops humilis* L., Methanol extract, Secondary metabolites, Water extract

INTRODUCTION

Algeria has a rich flora, and researchers have wide knowledge of their native medicinal plants. Medicinal plants represent an important constituent of flora and are broadly distributed in several regions of Algeria.

Chamaerops humilis L., var. argentea, is an Arecaceae widely distributed in all Mediterranean countries particularly in Algeria which has been located especially in coastal, arid and semi-arid regions. In addition, its also present in other places on the world used in ornement (Hirai *et al*., 1986). In the family of Arecaceae the genus of Chamaerops is represented only by humilis species.

In traditional medicine, *C. humilis* has been used in several diseases particularly for digestive system disorders (Benmehdi *et al*., 2012) and in gallstones and liver diseases (El-Hilaly *et al*., 2003). In addition, some compounds of extracts of Chamaerops have been reported to possess antioxidant (Benahmed-Bouhafsoun *et al*., 2013, Ben-

messaoud *et al*., 2014, Gonçalves *et al*., 2018) hypolipidemic and hypoglycemic (Gaamoussi *et al*., 2010), antilithic (Beghalia *et al*., 2008) urinary antiseptic and anti-inflammatory (Bellakhdar *et al*.,1991) and anticholinesterase (Gonçalves *et al*., 2018) activities. Besides its medicinal importance, Chamaerops presents an ecologically important role, it provides protection against erosion (Khoudali *et al*., 2014) since it allows attachment of the soil and fight against desertification.

Previous studies have shown that *C.humilis* contained phenolic compounds, including flavonoids, tannins, saponins, coumarines, quinons (Benahmed-Bouhafsoun *et al*., 2013) sterols and terpenoids (Hasnaoui *et al*., 2013). Herein, the anticholinesterase and antioxidant activities, and total phenolic and flavonoid contents of leaves and fruits methanol extracts of *C. humilis* L. were investigated in detail.

MATERIALS AND METHODS

The plant material (*Chamaerops humilis* L.) was collected from western Algeria (Oran).

Preparation of the extracts: Three g of dried samples were soaked in 50 mL of 80% aqueous methanol and ultrapure water at 60°C for 30mn under continuous reflux. The extracts were filtered through nylon mesh. The extraction was repeated two times. The filtrates obtained were dried using a rotary evaporator at 30ºC.

Instruments and Chemicals: A microplate reader (BioTek Power Wave XS, USA) and UV spectrophotometer were used for the antioxidant and anti-Alzheimer activities assays. All the chemicals using in antioxidant and anti-Alzheimer activities were analytical grade and purchased from Merck (Germany), Sigma (Germany), Aldrich (Germany), Sigma–Aldrich (Germany), Applichem (Germany), Fluka (Germany).

Determination of total phenolic and flavonoid content: The concentrations of phenolic content in the crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs) (Slinkard and Singleton, 1977). Eight microlitre of the extract in methanol was added to 176 µL of distilled H_2O and 4 µL of Folin-Ciocalteu's Reagent, and mixed. After 3 min, 12 µL of Na_2CO3 (2%) was added, and the mixture was shaken for 2 h at room temperature. The absorbance was measured at 760 nm. Total phenolic content was calculated according to the following equation.

Absorbance = 0.0296 pyrocatechol (µg) + 0.0177 (R^2 = 0.9952) …….Eq.1

Total flavonoid content of the extracts was measured according to the method described by Moreno *et al*. and results were expressed as quercetin equivalents (Moreno *et al*., 2000).Twenty microliter of the solution (1 mg of extract in methanol) was added to 4 µL of 10% aluminium nitrate, 4 µL of 1 M potassium acetate and 172 µL of methanol. After forty minutes, the absorbance was measured at 415 nm. Total flavonoid content was calculated according to the following equation:

Absorbance = 0.2956 quercetin (µg) - 0.1394 (R^2 = 0.9922) …….Eq.2

Antioxidant activity of the extracts: ABTS cation radical scavenging activities (Re *et al*., 1999), and cupric reducing antioxidant capacity (CUPRAC) (Apak *et al*., 2004) methods were used to determine antioxidant activities of the extracts.

ABTScation radical decolorization assay: 7 mM ABTS in distilled water was added to 2.45 mM potassium persulfate to produce ABTS• and solution was kept in the dark at room temperature for 12-16 h. The solution was diluted with ethanol until an absorbance of 0.700 ± 0.025 at 734 nm was obtained. 40 µL of each sample solution at different concentrations was added to 160 µL of ABTS• solution. After 30 min, the percentage inhibition was measured 734 nm. The scavenging capability of $ABTS^{\bullet+}$[15] was calculated according to the following equation:

$ABTS^{\bullet+}$ scavenging effect (Inhibition %) = $\frac{A_{control} - A_{sample}}{A_{control}} \times 100$ ….Eq.3

Cupric reducing antioxidant capacity (CUPRAC) method: Aliquots of 61 µL of $CuCl_2$ (1.0 × 10−2 M), 61 µL of neocuproine (7.5 × 10−3 M) and 61 µL of NH_4OAc buffer (1 M, pH 7.0) solution were stirred, x µL sample solution (2.5, 6.25, 12.5, and 25 µL) and (67 − x) µL distilled H_2O were added until the final volume 250 µL was attained. The tubes were left to standing for 1h. The absorbance was measured at 450 nm against a blank reagent (Apak *et al*., 2004).

Anticholinesterase activity of the extracts: The acetyl-cholinesterase and butyryl-cholinesterase inhibitory activities of the extracts were tested by using the method developed by Ellman *et al*. (1961).

Ten microliter of sample solution and 20 µL BChE (or AChE) were added to 150 µL of 100 mM sodium phosphate buffer (pH 8.0). Solution were stirred and incubated at room temperature for 15 min, then 10 µL of DTNB was added to mixture. The reaction was started by the addition of 10 µL butyrylthiocholine iodide (or acetylthiocholine iodide). Microplate reader (BioTek Power Wave XS) at 412 nm was used to monitor the hydrolysis of these substrates.

$$\text{Inhibition \%} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

…….Eq.4

For antioxidant tests the final concentrations of each method are 10, 25, 50 and 100 µg / mL.

For anticholinesterase activity, final concentration

is 200 µg / mL.

Statistical Analysis: All measurements were carried out in triplicate and results were expressed as mean± standard deviation (SD). The statistical significance was evaluated using a Student's *t*-test, *p* values <0.05 were considered as significant.

RESULTS AND DISCUSSION

Content of phenolic compound and flavonoids: Results from evaluation of total flavonoid and total phenolic contents of methanolic and water extracts prepared from leaves and fruits were determined as quercetin and pyrocatechol equivalents, respectively and are shown in Table 1. The total phenolic content was detected to be higher in water extract of *C. humilis* leave with 72.3 ± 0.97 µgPEs/mg of extract and was considerably decreased in methanol extract 62.16 ± 1.15 µgPEs/mg of extract of the same sample. Furthermore, the fruit of both methanol and water extracts showed similarity of phenolic content, 57.09 ± 1.41 µgPEs/mg and 58.78± 1.19 µgPEs/mg respectively, these values were found to be lower than the leave samples.

The results of quantitative analysis of flavonoid (Table 1) indicated that a higher content of flavonoids was obtained in the methanol extract of *C. humilis* leave with 7.69± 0.17µg in terms of quercetin equivalents/mg of extracts, followed by water extract 6.95±0.22 µgQEs/mg extract. However, in fruit both methanol and water extracts registered much lower amounts of flavonoids with 6.18±0.05µg/mg and 6.57±0.33µg/mg, respectively. This difference was less pronounced. In previous studies, the presence of phenolic substances including flavonoids was reported (3.70 mg/g) in *C. humilis* L. (Khoudali *et al*., 2014).

Furthermore, flavones C-glycosides and tricin were identified in *C. humilis* leaves (Williams and Harborn, 1973). Rutin, apigenin and luteolin were also revealed in leaves and fruits extracts of *Chamaerops humilis* L. (Bouhafsoun *et al*., 2018).

The results of this study highly imply that phenolic components are important components in Chamaerops and some of its biological properties could be attributed to the presence of these constituents. The reason for the observed variance in content of phenolics may be due to the variation in accumulation of these substances in several tissues and cells as epidermal cells of leaves, vaxes and external surfaces of fruits (Hutzler *et al*., 1998; Kuppusamy *et al*., 2016).

Antioxidant properties of different extracts: In the current study, the antioxidant activity of *C. humilis* extracts were determined in view of the CUPRAC and ABTS+ radical scavenging capacity of fruit and leave methanol and water extracts. The obtained results as can be seen in Figure 1 and Table 2 indicate that the highest ABTS scavenging activity was demonstrated by methanol leave extracts (50.11% in ABTS assay), which was richer in flavonoid contents. This activity was two times higher than in methanol fruit extracts, whereas the other remaining water extracts had comparatively low activity (20.35%).

Comparison between solvent extraction showed that the Methanol extracts have good activity against ABTS compared to the water solvent extracts (Figure 1).

Table 1. Total phenolic and flavonoid contents and anticholinesterase activity of *Chamaerops* extracts[a].

Extracts	Phenolic content (*µ*g PEs/mg extract)[b]	Flavonoid content (*µ*g QEs/mg extract)[c]	Inhibition % Against AChE	Inhibition % Against BChE
Fruit Methanol	58.78± 1.19	6.18± 0.05	NA	31.65 ± 0.37
Fruit Water	57.09 ± 1.41	6.57 ± 0.33	NA	30.19 ± 0.56
Leave Methanol	62.16 ± 1.15	7.69 ± 0.17	NA	NA
Leave Water	72.30 ± 0.97	6.95 ± 0.22	NA	NA
Galanthamine[d]	-	-	86.25 ± 0.31	77.41 ± 0.16

[a] Values are means ± S.D. of three parallel measurements, $p<0.05$, significantly different with Student's *t*-test. [b] PEs, pyrocatechol equivalents; [c] QEs, quercetin equivalents; [d]Standard drug; NA: Not active

Table 2. Cupric reducing antioxidant capacity of *C. humilis* extracts.

Extracts	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Fruit Methanol	0.139±0.011	0.145±0.028	0.183±0.014	0.243±0.009
Fruit Water	0.088±0.008	0.109±0.007	0.203±0.097	0.531±0.500
Leave Methanol	0.128±0.025	0.169±0.004	0.265±0.025	0.395±0.002
Leave Water	0.091±0.009	0.121±0.006	0.164±0.016	0.301±0.034
BHT	1.456±0.101	2.723±0.304	3.820±0.084	3.940±0.142
TOC	0.454±0.026	1.179±0.093	1.965±0.181	3.260±0.015

Values expressed are means ± S.D. of three parallel measurements and values given as absorbance

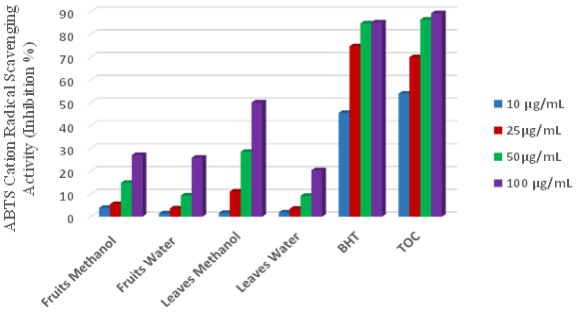

Fig. 1. *ABTS cation radical scavenging activity of C. humilis L. extracts, α-TOC and BHT. Values are means ± S.D., n=3. p<0.05, significantly different with Student's t-test.*

Againts CUPRAC, the results showed that the cupric ions (Cu^{2+}) reducing capacity of the water extract of *C. humilis* fruit was twice higher than the methanol extract tested (0.531 and 0.243) respectively, but lower than synthetic antioxidants α-tocopherol (α-TOC) and butylated hydroxytoluene (BHT) (Table 2). Although, the all the leave extracts exhibited moderate activity in CUPRAC method at four different concentrations.

The results of this study were compared with those obtained by previous research, which determined the antioxidant properties of *C. humilis* leaves by the DPPH method. They reported an IC_{50} of 180.71 ± 6.6 µg mL^{-1} in a DPPH assay (Benahmed-Bouhafsoun *et al*., 2013). The total antioxidant activity of several Arecaceae was evaluated based on scavenging activity of DPPH free radicals, among them the date fruits of *Phœnix dactylifera* of Oman showed an inhibition value about 65% (Singh *et al*., 2012), and 67% in the leaf extract of *Hyphaene thebaica* (Mohamed *et al*., 2009).

In general, *C. humilis* leaves have a better antioxidant profile. The result was related with their flavonoids and phenolic content.

Anticholinesterase activity: The deficit of acetylcholine in the brain could be causes the Alzheimer's disease (AD) which is the most common form of neurodegenerative disease (Bachman *et al*., 1992). Therefore, AD can be treated with both two form of cholinesterase, acetylcholinesterase (AChE) and butrylcholinesterase (BChE) (Jaen *et al*., 1996). This study evaluated the anticholinesterase activity of *C. humilis* using the Ellman method, Galantamine was used as positive control which is used for Alzheimer's disease. The results obtained from this study showed that all the Chamaerops fruit extracts exhibited moderate inhibition against butyrylcholinesterase enzyme (BChE) at 200 µg/mL concentration. As shown in Table 1, it showed 31.65% for the methanol and 30.19 % for the water extract inhibitory activity against BChE. Some studies have reported the anti-cholinesterase activity due to the presence of active compounds including flavonoids, tannins, alkaloids, quinins, stilbens and xanthons (Pinho *et al*., 2013, Silva *et al*., 2014; Gonçalves *et al*., 2018).

Contrariwise, no extracts from the leave have shown any inhibition against acetyl and butyrylcholinesterase enzyme. Also, the methanol and water fruit extracts displayed no activity on acetylcholinesterase at 200 µg/mL.

Conclusion

In this study, extracts of *Chamaerops humilis* L., demonstrated that they not only possesses antioxidant and radical scavenging activities but also exhibit inhibitory potential against acetylcholinesterase. Furthermore, the fruit extracts exhibited promising anticholinesterase activity, constituting the first report of anticholinesterase activity to extracts from Algerian *C. humilis*. Consequently, leave and fruit *C. humilis* that are rich in phenolics and flavonoids and promising for the development of safe food products and can be used for the future therapeutic medicine.

ACKNOWLEDGEMENTS

This work is supported by High Teaching and Scientific Research Ministry www.mesrs.dz

The authors are greatful to Dicle University Faculty of pharmacy for opening its laboratory facility.

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