Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

PHYTOCHEMICAL COMPOSITION AND IN VITRO ANTIOXIDANT ACTIVITY OF *CHAMAEROPS HUMILIS* L. EXTRACTS

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Received: 23 Apr 2013, Revised and Accepted: 06 Jun 2013

ABSTRACT

Objective: The current study aims to evaluate the phytochemical content and the antioxidant activity of methanol extracts of *Chamaerops humilis* L.

Material and Methods: *Chamaerops humilis* L. leaflets, rachis and roots were powdered and extracted with methanol and they were screened by various chemical tests and TLC studies.

The total phenolic and flavonoid contents were assessed by spectrophotometric method and the antioxidant activity was estimated using 2, 2-di-phenyl-1-picrylhydrazyl (DPPH) as a free radical scavenger.

Results: The results revealed the presence of flavonoids, tannins, coumarins, saponins and quinons. Alkaloids was not detected in any parts extract under study. The flavonoids content varied from 40,7±0,53 mg/g and 38,1±0,9 mg/g, whereas total phenolic content is 26 to 28,7 mg /g in all parts. The IC50 value for DPPH radical with methanolic extracts of *Chamaerops humilis* L. was found to be 180,71µg ml^{-1}.

Conclusion: These results suggest that methanol extracts have good potential as sources of different bioactive compounds and antioxidants.

Keyword: *Chamaerops humilis* L., Screening, Phenolic compounds, Flavonoids content, Antioxidant activity.

INTRODUCTION

Polyphenolic compounds have been shown in recent years to be of vital significance to mankind as well as to plants. They have been strongly implicated as active contributors to the health benefits of beverages such as tea and foods such as fruit and vegetables.

In addition, it has been found out that plant having polyphenolic compounds such as flavonoids possess antioxidant activity [1]. Some evidence suggests that the biological actions of these compounds relate to their antioxidant activity [2]. Also, they have anti-inflammatory and anticancer properties, isolation and extraction of this compound in vivo (leaf, stem, fruit and root), can be exploited in medicine.

In Algeria, Arid and semi arid plants are good sources for the production of various types of secondary metabolites which make them resistant to various environmental stress scarcity of water, salinity, pathogens etc. They are also important for the primary metabolism of plants. These compounds include alkaloids, flavonoids, steroids, phenolics, terpens, volatile oils etc. Man has been exploiting these natural plant products for use in medicines, cosmetics, dyes, flavors and foods. Genus *Chamaerops humilis* L., subsp argentea, is endemic Arecaceae (Palmeae) of North-Africa, It covers a large area in the arid and semi-arid environment and presents an ecologically important role, it provides protection against erosion since it allows attachment of the soil and fight against desertification. It is present abundantly in the littoral of the country. It is characterised by adventitious roots, the leaves in range, palmate, rigid consist of leaflets and of rachis, this last is fine carrying spines along the edges, the leaflet is in range completely open or partially open, reaching 70 cm length and 80cm broad. The flowers are presented in dense, multifarious inflorescences. The fruit of *Chamaerops humilis* L. is a bay solitary, oblong yellow reddish with brown red.

On the anatomical and histological study, this species is characterized by the presence of cellulosic and lignified tissues with syringyl lignin being the predominant structure [3, 4]. In many countries around the world plants are used in folk remedies; therefore this genus is of great importance in traditional medicine which it is a preventive treatment against prostate problems and acts as a natural disinfectant of the urinary tract. In vitro, the aqueous extract of C. *humilis* L. Inhibits the growth of calcium oxalate calculi which caused the urolithiasis disease [5]. Recently, it has been shown that the aqueous leaf extract of *C. humilis* L. has a hypoglycemic and hypolipidemic effects [6]. It seems not to be studied enough chemically contrary to other species of the same family. Taking into account the biological importance of the species and with an aim of its valorization, we made studies which highlighted the richness of the plant out of secondary metabolites. In the present investigation, phytochemical screening of methanolic extracts of different parts of *C. humilis* revealed the presence of phenolic and flavonoids compounds. Hence the present study was designed to evaluate the antioxidant activity of *Chamaerops humilis* L.

MATERIALS AND METHODS

Plant material and Preparation of extracts

The plant materials used for this investigation were developed, mature leaves (leaflets, rachis) and roots *Chamaerops humilis* L., Collected from the university of sciences and technology Mohamed Boudiaf of Oran (USTOMB) in March 2010. Plant materials were dried and milled into uniform powders using a knife crusher of the type RETCH provided with a mesh filter then stored carefully until use. 5 g of the dried powdered plant was soaked separately in 100 ml of methanol (98%). The extracts were filtered through nylon filter. The collected filtrates were dried under vacuum using a rotary evaporator, the extraction was repeated twice. The resulting residue was redissolved in methanol and used for determination of phenolic and flavonoid compounds and antioxidant activity.

Total phenolic content determination

The concentration of polyphenol in the methanol extract of Chamaerops was determined using the Folin–Ciocalteu method [7]. 100 µl of the sample were added to 150 µl of Folin-Ciocalteu and aqueous sodium carbonate Na_2CO_3 (2%). The mixture was incubated for 1h and the total phenols were determined by spectrophotometer (UVIKON UV/VIS) at 765 nm. The standard curve was prepared using 0,1µg/m - 0,2µg/ml - 0,3µg/ml - 0,4µg/ml-0,5µg/ml of gallic acid solutions in methanol. Total phenol values are expressed in terms of gallic acid equivalent (mg. g^{-1} of dry mass), which is a common reference compound.

Total flavonoids determination

The flavonoid content was measured using the colorimetric method adapted by Kim (2003) [8]. The methanolic extract (500 µl) was mixed with 1500 ml of distilled H_2O and 150 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of a 10% $AlCl_3$ solution was added. About 500 µl of 1 M NaOH were added to the mixture, the intensity

of pink color was measured at 510 nm using a UVIKON UV/VIS spectrophotometer. The level of flavonoid concentration was calculated using catechin as a standard. Flavonoid content was expressed as mg catechin equivalents per g of dry extract.

Phytochemical Screening

Flavonoids: Aliquots of methanolic extracts were spotted on TLC plates (silica gel 60 F_{254} nm, aluminium support, Merck) and developed in ethyl acetate/formic acid/acetic acid/water, 100:11:11:26 (V/V) mobile phase. Visualization of the flavonoids was achieved by spraying the sheets with Neu's reagent. [9] Plate was observed under UV spectrum at 254 and 365 nm before and after spraying reagent.

Coumarins: Two grams (2g) of plant powder are mixed with 10ml of chloroform. After a heating of a few minutes and a filtration, the chloroformic extracts were subjected to a TLC with fluorescence, in which the mobile phase: Toluene/ethyl acetate/ 90:10 (V/V) [10]. The visualization of the chromatogram was made at 365nm after pulverization with Neu's reagent.

Alkaloids: Two grams (2g) of plant powder are added to 100ml methanol 50% and stored all night, the extracts were filtered and evaporated. The residues were included in a few ml of pure methanol. Extracts were spotted on a TLC plate (silica gel 60F_{254} nm, aluminium support, Merck) and developed in ethyl acetate/methanol/ammoniac, 90:10:10 (V/V) mobile phase. TLC was observed under UV spectrum at 365nm after spraying with Dragendorff's reagent [11]. The appearance in visible light of orange spots indicated the presence of alkaloids.

Tannins: 1,5 g of plant powder were added to 10ml of methanol 80%. After 15 minutes of agitation, the extracts were filtered. The addition of ferric chloride ($FeCl_3$)1% makes it possible to detect the presence of tannins. The blue color was observed for gallic tannins and green black for catecholic tannins [10].

Free quinons: One gram (1g) of plant was added to 20 ml of oil ether. After agitation and a rest of 24h, the extracts were filtered and concentrated with the rotavapor. The presence of free quinons is confirmed by the addition of some drops of NaOH 1/10, when the aqueous phase transfers into the red.

Saponins: About 0,5 g of the plant extract was vigorously shaken with 3 ml of distilled water in a test tube. Frothing, which persist was indicated as a preliminary evidence for the presence of saponins.

Determination of antioxidant activity

DPPH Analysis

The rapid evaluation of antioxidant activity was determined by TLC method. The silica gel 60 F_{254} (aluminium support, Merck) plates were dried and sprayed with 0,2% of 2,2-diphenyl-2-picryl-hydrazyl (DPPH) in MeOH, as an indicator. The presence of antioxidant compounds was detected by yellow spots against a purple background [12].

DPPH Radical Scavenging Activity

DPPH radical scavenging activities of all the fractions were determined by the method of Buenger [13]. with some modification. Initially, 2ml of the extract fractions at a concentration of 10, 20, 30, 40, 50, and 100 µg/ml respectively, was mixed with 2 ml of methanolic solution of 50µM DPPH. The reaction mixture was kept in dark at room temperature for 60 minutes. The control contained all reagents except the extract fraction while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 515nm using a spectrophotometer (UVIKON UV/VIS). The experiment was done in triplicate. Ascorbic acid was used as a standard control. The DPPH radical scavenging activity (%) of the sample was determined by the following equation**:**

DPPH scavenging effect (%) = $[A_{DPPH}-A_{extract} / A_{DPPH}] \times 100$

Where: A_{DPPH} is the absorbance value of the DPPH blank solution

$A_{extract}$ is the absorbance value of the sample solution

The IC50 value is the concentration of an antioxidant to quench 50% of DPPH free radicals in the reaction mixture under the assay condition. It was calculated graphically using a calibration curve in the linear range by plotting the extract concentration is the corresponding scavenging effect. A low IC50 value represents a high antioxidant activity.

Statistical analysis

Correlation between the antioxidant activity and total phenolic contents was carried out using the correlation and regression in the Excel program (Microsoft Excel 2007).

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the methanol extracts of *Chamaerops humilis* L. (Table 1) revealed the presence of flavonoids, tannins, quinons, coumarins and saponins in all the parts, except roots which contain few flavonoids

Table 1: Phytochemical screening of *Chamaerops humilis* L.

Plant Parts	Leaflets	Rachis	Roots
Total phenols	+	+	+
Total flavonoïds	+	+	+
Tanins	+	+	+
Quinons	+	+	+
Saponins	+	+	+
Coumarins	+	+	+
Alkaloids	–	–	–

+ : Present ; - : Absent

Rachis and leaflets showed a similar phytochemical composition. All the extract parts shown on the chromatograms gave a negative Dragendorff reagent so there is an absence of alkaloid compounds. Detection of coumarins by the TLC method after observation in UV-365 nm, revealed fluorescent brown stains. Indeed, these spots correspond according to Wagner and Bladt [14] to furano- and pyranocoumarines. The presence of tannins in all parts of extracts was confirmed by the reaction with ferric chloride who gives a color blue-black, characteristic of gallic tannins. As for epicatechic tannins, it was revealed in drupes of *Chamaerops humilis* L. and in leaves of *Phoenix canariensis* and *Livistona chinensis* [15]. Tannins are reported to exhibit antiviral, antibacterial, anti-tumoral activities. It was also reported that some tannins are used as diuretic [16].

Free quinons were also found in all parts of *Chamaerops humilis* L. since the reaction to NaOH 1/10 gives a red color characteristic of quinons. The existence of quinons in the roots is probably due to their role of defenses against the micro-organisms [17]. Saponins which are responsible for numerous pharmacological properties [18] were also detected in *C. humilis* parts extract. Saponins are known to produce inhibitory effect on inflammation [19]. It has been observed that many flavonoids and tannins exhibit anti-inflammatory effects [20]. It can therefore be suggested that the presence of the reported phytochemical constituents including the flavonoids and tannins in C.humilis may have contributed to the anti-inflammatory and antioxidant activities. Since the preliminary phytochemical screening showed the presence of a considerable amount of polyphenolics compounds which are the major phytoconstituents behind the antioxidant activity, so it is necessary to perform thin layer chromatography for their further confirmation.

For flavonoids, we characterized them with a specific reagent Neu. Several different fluorescence stains were observed on TLC, after the revelation and visualization at UV- 365 nm. For every specific color of spot with Rf, we made an assignment with a type of compound.

A densely yellow-green band was seen both in leaflets and rachis having an Rf of 0,46 which correspond according to Wagner and Bladt [14] to rutin. For flavonoids leaflets were seen to possess three orange bands with one band (Rf 0,62) having a dense orange color

after spraying, which correspond to luteolin. Leaflets *C. humilis* also showed the presence of densely yellow-orange band with Rf value 0,26, it corresponds according to Wagner and Bladt [14] to quercetin. This compound has anti-inflammatory, antioxidant and anticancer properties [21]. The blue stains Rf (0, 56) obtained in the same chromatogram indicates presence of phenolic acids in only leaflets and roots, especially *p*-hydroxybenzoïc acid which was found in the high content ester binding in *C. humilis* cell wall [4], It presence in roots can contribute to defense against pathogenic microorganisms [22]. The presence of *p*-hydroxybenzoic acid in many species of Arecaceae can be used as a biomarker chemotaxonomic of this family [23].

The results of TLC corroborate the presence of phenolics compound and flavonoids which comply with the results of preliminary phytochemical screening. The intensity of spots color obtained was comparatively more intense for leaflets. So it is understood that more amounts of flavonoids are contained in leaflets than roots *Chamaerops humilis* L. Also, the intensity of colouring of the separate products is proportional to their concentrations.

Some of these flavonoids such as tricin, and glycosylflavone were identified in leaves of some Arecaceae species as *Chamaerops humilis*, *Rhapis humilis, Phoenix canariensis*, *Phoenix dactylifera* and *Washingtonia filifera* [24, 25], these compounds were used as chemosystematic markers of monocotyledonae, including the Palmae [25].

Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruits and vegetables.

Total phenolic and flavonoids content

Polyphenols are plant secondary metabolites and are very important by virtue of their antioxidant activity by chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversions into reactive oxyradicals. Table 2 report the total phenolic compounds and flavonoids in fractions expressed as gallic acid equivalents and catechin equivalent respectively.

Table 2: Total phenolic and flavonoid content of *Chamaerops humilis* L.

Plant Parts	Total phenolic Content (mg/g)	Total Flavonoid Content (mg/g)
Leaflets	26,8± 0,41	40,7±0,53
Rachis	28,7±0,44	38,1±0,90
Roots	26±0,50	20,04±0,62

The results show a similar total phenolic content in roots and leaflets and a small difference between rachis and other parts. This is the first report on the chemical content of this plant species. The content of these compounds (mg/g) in terms of gallic acid equivalent (standard) varied from 26 to 28,7 mg /g in all parts. Roots contained phenolic compounds 26± 0,5 mg /g, whereas, rachis contains 28,7±0,44 mg/g

DPPH radical scavenging activity

In order to evaluate the antioxidant activity of choosing leaves (leaflets and rachis) of *C. humilis*, DPPH assay was applied and the results are presented in Table 3.

The extraction yield of methanol extracts of the powdered leaves of *Chamaerops humilis* L. was 20, 6% (W:W). To the best of our knowledge, there is no information available in the literature on the antioxidant activity of the leaves of the *Chamaerops humilis* L. specie under investigation; this is why we conducted this study. Percentage inhibition of DPPH and IC50 are parameters widely used to measure antioxidant and free radical scavenging [30]. Results show that the inhibition of scavenging activities of the *C. humilis* extract for DPPH is 65% at a concentration of 0,1 mg/ml, and the IC50 value obtained to quench 50% of DPPH free radicals is 180,71 µg/ml.

Table 3: IC_{50}, and EC_{50} values of methanol extract leaves of *Chamaerops humilis* L.

Plant Part	IC_{50}	EC_{50}
Leaves	180,71±6,6	7,22±0,91
Ascorbic acid	159,5±4,89	6,38±0,18

IC_{50}: antiradical activity (µg ml^{-1})

EC_{50} : efficient concentration (µg sample / µg DPPH)

The total antioxidant activity of some Arecaceae species was assessed based on scavenging activity of DPPH free radicals, among them the leaf extract of *Hyphaene thebaica* (palm of Egypte) obtained a same inhibition value 67% [31], and 65% in date fruits of *Phoenix dactylifera* of Oman [32]. The "efficient concentration" or EC_{50} value is another parameter introduced for the interpretation of the results from the DPPH method. It is defined as the concentration of the substrate that causes 50 % loss of the DPPH activity (Table 3). Its value was found to be 7, 22 µg sample / µg DPPH. And the correlation between the content of total phenolics and the antioxidant index was found to be $R^2 = 0.98$.

These results indicated that methanolic extracts of *Chamaerops humilis* have a noticeable effect on scavenging free radical. However, the scavenging effect of ascorbic acid a commercial antioxidant as standard is slightly higher than the extracts of Chamaerops

We suggest that *Chamaerops humilis* L. is endowed with antioxidant phytochemicals and could serve as a base for future drugs. Antioxidant activities due to the presence of some bioactive compounds like phenolics including flavanoids. It has been reported that property of date fruits of *Phoenix dactylifera* from different cultivars grown in Algeria [28] and in Oman [32] is quietly higher than that of *C. humilis* extract. In general, phenolic compounds were commonly found in plants and have reported several biological activities including potent antioxidants and free radical scavengers apart from the primary defense role [33]. Epidemiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart diseases [34] and in raising the hepatoprotective activity [35].

CONCLUSION

The phytochemical analysis performed on the various extracts of *Chamaerops humilis* L. from Algeria indicated the presence of phenolics compounds, flavonoids, quinons, tannins, saponins and coumarins. Contents of polyphenols and flavonoids which were determined in the leaflet, rachis and roots of the specie differ from one sample to another. The reported results show that the methanol extract of *C. humilis* contains the highest amount of flavonoids compounds and exhibits a great antioxidant activity through the scavenging of free radicals which participate in various pathophysiology diseases including ageing. Further studies are needed to determine antimicrobial activity. The various phytochemical compounds detected are known to have beneficial importance in industrial and medical sciences. We believe these compounds in *C. humilis* leaves could be harnessed for medicinal sciences utilization.

ACKNOWLEDGEMENTS

We would like to thank Pr. Marouf A., Es Senia University and Dr. Haddi R., USTOMB University, for their timely help during this investigation.

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