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Phytochemical study of four leaves extracts of Chamærops humilis L. from the region of Al-Hoceima, Morocco

Mohamed El bastrioui [1], Khadija Haboubi [1], Ahmed Chetouani [2], Belkheir Hammouti [2,3], Asep Bayu Dani Nandiyanto [4]

[1] *Laboratoire des sciences de l'ingénieur et applications (LSIA)" Adresse: Ecole Nationale des Sciences Appliquées Al Hoceima BP 03, Ajdir Al-Hoceima*

[2]*LCAE, Faculty of Sciences, University Mohammed Premier, B.P. 724, 60000 Oujda Morocco*

[3]*Centre de Recherche (CREHEIO), Ecoles des Hautes Etudes d'Ingénierie, EHEI, 60000 Oujda, Morocco*

[4] *Universitas Pendidikan Indonesia, Jl. Dr. Setiabudi no 229, Bandung 40154, Indonesia*

** Corresponding author:*
mohamedelbastrioui@gmail.com
ahmedchetouani70@hotmail.com

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Abstract

The objective of this study was to compare the relative yields of two extraction methods (maceration and soxhlet extraction) using solvents of different polarities and to quantitively and qualitatively assess the secondary metabolite contents of the Chamærops humilis L. plant. In the experiments, we used the maceration and the extraction processes using soxhlet. Several solvents were used, such as diethyl ether, ethyl acetate, ethanol, and water. For the phytochemical screening, we relied on the color and precipitation tests, and for the quantitative determination of the content of total phenol, flavonoids, and flavonols, we used conventional methods. The results showed that the phytochemical screening of the various extracts showed the presence of the majority of metabolite dispersed between the four extraction solvents with exception of alkaloids and proteins which are absent in all the extracts. For the total phenol content, it was in large amounts in the ethanolic extract for the maceration technique and a large amount in the aqueous extract for the soxhlet extraction. In addition to flavonoids and flavonols, experimental tests have shown that ethanol has well extracted these two types of components. The plant Chamærops humilis L. from our region has shown the presence of a high concentration of total phenol and more precisely flavonoids, which can prove its use as an antibiotic for a lot of diseases.

Keywords: *Chamærops humilis L; soxhlet extractor; maceration; phytochemical screening; total phenol; flavonoids.*

1. Introduction

Medicinal plants have been very useful since ancient times for curing human diseases due to the presence of phytochemicals [1]. These are naturally occurring biochemicals called secondary metabolites and are the end products of primary metabolites such as carbohydrates, amino acids, chlorophyll lipids, etc. Many plants are well-known for their secondary metabolic potential. These compounds are synthesized in leaves, bark, fruits, stems, and roots. They contribute to giving plants their characteristic colors, flavors, smells, and textures [2]. The Arecaceae family includes 200 genera and 3000 species. Chamaerops humilis L. is a member of this family, it is qualified as a medicinal plant by some [3]. It is also called dwarf palm or "doum", and it can reach between an average height of 1 and 1.5 m. Chamaerops humilis L. is very widespread in North Africa and the European Mediterranean coast [4].

Some components of this plant are consumed as food and used in traditional medicine. The husk (Higa) is consumed in Southern Spain, the fruits in Morocco, and the yellow suckers in Italy [5]. In Algeria, the spadices and the heart of the palm are used to treat several disorders of the digestive tract [6]. The leaves are used in Morocco and Algeria for the treatment of diabetes [6,7]. Several phytochemical studies have reported the presence of Tannins, flavonoids, saponins, sterols, and terpenoids, which may explain its pharmacological effects [8-12]. Other works have shown that extracts of Chamaerops humilis L have antioxidants [9,12-15], anticholinesterase [13,15], hypolipidemic and glycemic [16], antilithic [17], antiseptic and anti-inflammatory [18], antibacterial [19], and anticorrosion properties [20]. Generally, the activities of extracts obtained from a plant may be subject to change according to variations in chemical composition [21-28]. In addition, the difference in activities may be due to the geographical origin, the locality, the climatic conditions, and the time of harvest of the collected plant material [29-30]. Here, we decided to study the Chamærops humilis L. plant, which is very abundant in our region of Al-Hoceima in the North of Morocco. Indeed, we aim to carry out a phytochemical study of four extracts (i.e. diethyl ether, ethyl acetate, ethanol, and water). The aim is to characterize the secondary metabolites that will explain the therapeutic effects of this plant.

2. Materials and methods

2.1. Collection of samples.

The leaves of Chamærops humilis were cut by a special chisel and collected in March 2021 at Al-Hoceima National Park (Rif) in northern Morocco, at an altitude of 380 m and geometric coordinates 35° 13'5' 'N and 3° 59'6' 'W. The fresh leaves were packed in a polyethene bag and then transported immediately to the laboratory.

2.2. Preparation of samples.

The fresh leaves of the plant were thoroughly cleaned and spread out to dry in a place in the laboratory with good ventilation for three weeks. The dry leaves were then ground using a grinder (Moulinex, France) and stored in glass vials protected from light and moisture for subsequent analysis.

2.3. Preparation of extracts.

2.3.1. Soxhlet's method.

A quantity of 20 g of vegetable powder was placed in a cartridge and then brought into contact with four solvents in increasing order of polarity: diethyl ether, ethyl acetate, ethanol, and water. The extraction was stopped when the liquid surrounding the cartridge becomes colorless, indicating that the solvent was no longer extracting anything from the solid. After each extraction, the solution obtained was concentrated with a rotary steamer until a solid was obtained which was then used for phytochemical tests.

2.3.2. Maceration method:

20 g of powder was put in contact with 100 mL of diethyl ether, the whole was homogenized by manual shaking for 10 min, the mixture was then filtered under vacuum on a Büchner, and the filtrate obtained named etheric filtrate 1. The same procedure was repeated on the residual for the second and third time, these three filtrates were combined and concentrated at 25 mL on a water bath. After depletion in diethyl ether, the residual marc was dried and then recovered in 60 mL of ethanol, 10 min of homogenization by manual stirring made it possible to obtain the first ethanolic filtrate, the operation was repeated with 60 mL of Ethanol, after filtration, the two filtrates were combined and concentrated to 25 mL on a water bath.

To prepare the aqueous extract, we infused 10 g of each powder in 50 mL of distilled water, for 15 min, the extracts were then filtered through filter paper.

2.3.3. Determination of moisture content:

The moisture content of plant leaves was determined at 105°C (28). A mass of fresh leaves was steamed for 1.5 h. Three replicates were performed, the average of which would represent the moisture level. Then, we measured using equation (1) for determining moisture content (*R%*):

$$R\% = \frac{m_{sec}}{m_{fraiche}} \times 100 \quad (1)$$

where m_{sec} is the mass of dry matter in grams and $m_{fraiche}$ is the mass of the fresh plant sample in grams.

2.3.3. Extraction yield:

For the decocate or the solvent extract, regardless of the method followed, the extraction yield (ρ) is calculated by equation (2)

$$\rho = \frac{m_{ext}}{m_{éch}} \times 100 \quad (2)$$

where m_{ext} and m_{ich} are the mass obtained in the exit and initial/theoretical calculation, respectively.

2.4. Phytochemical screening.

The phytochemical analysis of the extracts and the infused was carried out by standard methods described in Sofowora [31].

2.5. Test for Alkaloids:

In a conical flask, one gram of Chamærops humilis powder was put and an ammonia solution was added (2 mL). It was left to rest for a few minutes to evaluate the free alkaloids. Chloroform (5 mL) was added to the hand-shaken conical flask and then filtered. The chloroform was evaporated from the crude extract in a water bath and Mayer's reagent (2 mL) was added. A cream-colored precipitate was obtained immediately which showed the presence of alkaloids.

2.6. Test for flavonoids.

Each extract (1 mL) was taken into a test tube and a few drops of diluted NaOH solution was added. An intense yellow color appeared in the test tube. It turned colorless upon adding a few drops of dilute acid which indicated the presence of flavonoids.

2.7. Test for Tannins

In the general test, to 1 mL of extract, a few drops of $FeCl_3$ are added, and a blue-black color or formation of a green precipitate indicates the presence of tannins. For catechetical tannins, 3 mL of each extract was evaporated to dryness. After adding 10 mL of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80° C for 20 min. The observation of a precipitate in large flakes characterizes the catechetical tannins. For gallic tannins, the previous mixture was filtered, and 0.5 mL of the filtrate was then saturated with sodium acetate. To this mixture 2-3 drops, 1% of $FeCl_3$ were added; the development of a blue-black tint indicates the presence of gallic tannins not precipitated by Stiasny's reagent

2.8. Saponins test.

A quantity (0.1 g) of the extract was boiled with 5 mL of distilled water for 5 min; the mixture was filtered hot, and the filtrate was used for two tests: In the emulsion test, a few drops of olive oil are added to 1 mL of the filtrate and shaken manually. The formation of an emulsion indicates the presence of saponins. In the foam test, 1 mL of filtrate was mixed with 4 mL of distilled water, the mixture was then stirred vigorously, the observation of a persistent foam after standing for 15 min indicates the presence of saponins.

2.9. Steroid test:

We used the Salkowski method. To 2 mL of extract were added 2 mL of Chloroform and 2 mL of concentrated sulfuric acid. The mixture was stirred well. The chloroform layer turns up a greenish-yellow fluorescence

2.10. Anthocyanin test

To 5 mL of each extract of the infused (5%) was added 5 mL of 10% sulfuric acid followed by NH_4OH base (5 mL). The presence of anthocyanin was confirmed if the color was increased by acidification and then turns blue purplish in a basic medium.

2.11. Protein test.

We used the Biuret test. To 3 mL of extracts were added 4% NaOH and a few drops of a 1% solution of $CuSO_4$. The purple or pink color appears.

2.12. Quinone test:

We used the Bornstraëgen test. An aliquot of residue dissolved in 5 mL of HCl was diluted to 1/5, heated in a boiling water bath for 30 min, and extracted with 20 mL of $CHCl_3$ after cooling. To the organic phase were added 0.5 ml of 50% NH_4OH. The appearance of a shade ranging from red to purple indicates the presence of quinones.

2.13. Cardiac glycosides test:

We used the Keller-Killani method. 1 mL of glacial acetic acid containing traces of $FeCl_3$ and 1 mL of concentrated sulfuric acid were carefully added to the extracts. A reddish-brown color forms at the junction of two layers and the top layer turns bluish-green in the presence of glycosides.

2.14. Mucilage test:

1 mL of decocted was put in a test tube. Then, 5 mL of absolute ethanol was added. Obtaining a flaky precipitate after shaking indicates the presence of mucilage.

2.15. Total phenol content

The total phenol content of chamærops humilis extracts was measured based on the method using Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as standard. A dose of 200 µL of the extract was placed in a test tube and then added 1 mL of Folin-Ciocalteau reagent (diluted 10 times). The mixture was stirred well and then allowed to stand in the dark for 5 min at about 23°C. 750 µL of a 7.5% sodium carbonate solution (Na_2CO_3) were added. After incubation for 2 hours, the absorbance was measured at 760 nm by a spectrophotometer (UV-1800 Shimadzu) against a blank. The calibration curve was prepared using gallic acid as a standard. After plotting the absorbance against the concentration, a linear relationship was used as a standard curve for the determination of the total phenol content of the samples. The results obtained were expressed in mg EQG/g_{ext}.

2.16. Total flavonoid content.

Based on the aluminum trichloride method described in the previous study (33), the total flavonoid content was determined using quercetin as a reference. 1 mL of each suitably diluted extract (1 mg/mL) was placed in 10-mL test tubes with 1 mL of aluminum trichloride (2% in methanol) and a drop of acetic acid. The mixture was incubated at room temperature for 40 min, and the absorbance was measured at 415 nm against a blank prepared with 1 mL of methanol and 1 mL of aluminum trichloride (2%) plus a drop of acetic acid. A standard range (5-100 µg/mL) based on quercetin was also prepared under the same conditions. The flavonoid content in the extracts was expressed in (mg EQ/g_{ext}).

2.17. Total flavonols content.

We used the method of Kosalec et al. [32] using aluminum trichloride with a slight modification to determine the content of flavonols in different extracts (34). In a test, tubes were introduced: 1 mL of distilled water, 0.1mL of $AlCl_3$, 1 mL of sodium acetate (50 g/L), and 1 mL of the extract, the mixture was vigorously stirred, then the whole was incubated in the shade at room temperature for 90 minutes. The absorbance was read at 440 nm. Under the same conditions above, the flavanols content was expressed in milligrams of quercetin equivalent per gram of dry weight of the plant (mg EQ/g_{ext}).

3. Results and discussion

3.1. Humidity level

The humidity is around 50%, half of the wet weight is water, three tests have been carried out (see Table 1). The condition of the soil, the climate, the age of the plants as well as the shelf life of the plant after harvest, are factors that can influence the values of the humidity level.

Table 1: percentage of moisture of chamærops humilis L. leaves

	the mass of fresh material (g)	the mass of dry matter (g)	water content (%)
First assay	7.54	3.72	49.34
second assay	6.39	3.22	50.40
third assay	5.74	2.76	51.11

3.2. The extraction yields.

The extraction yield was calculated based on the total weight of the powder. The results obtained are shown in Figure 1. Usually, the extraction yield increases with the increase in the polarity of the solvent, but it appears that the soxhlet extraction technique has a good yield, which confirms that the yields of the extracts depend on the extraction method

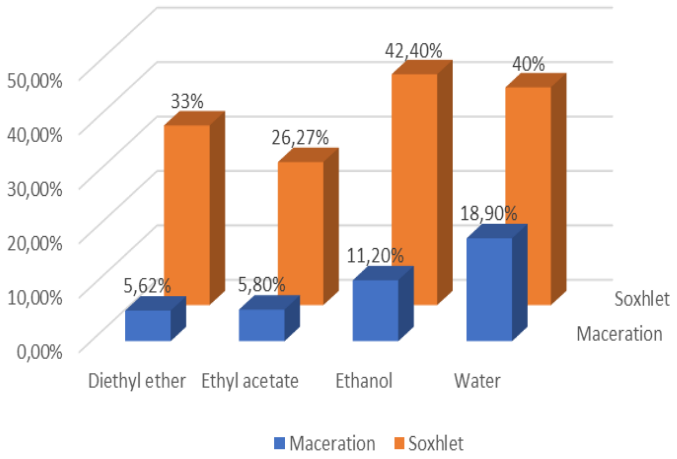

Figure 1. Extraction yield

3.3. Phytochemical screening:

The tests of the chemical groups were carried out, and the results are mentioned in Table 2. The table indicates the presence of the main secondary metabolites: in all the extracts, phenols and flavonoids were detected; while alkaloids, anthocyanins and proteins are absent. Steroids, saponins, and mucilage are only detected in the aqueous extract, while tannins, cardiac glycosides, and quinones are also detected in the ethanolic extract.

Table 2: phytochemical constituents of four solvents extracts of chamærops humilis L.

Chemical		Etheric extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
Phenols		+	+	+	+
Alkaloids		-	-	-	+
Flavonoids		+	+	+	+
Tannins	general	-	-	+	+
	catechetical	-	-	+	-
	galliques	-	-	+	+
Steroids		-	-	-	+
Saponins	Emulsion	-	-	-	+
	foam	-	-	-	+
Proteins		-	-	-	-
Cardiac glycosides		-	-	+	-
Quinones		-	-	+	-
Mucilages		-	-	-	+

3.4. Determination of total polyphenols:

The determination of the total phenol contents for the four extracts was reported in mg EAG/g_{ext}. The number of polyphenols was determined from the gallic acid standard solution calibration curve (see Figure 2). The results of the assay for the two extraction techniques are grouped in Table 3.

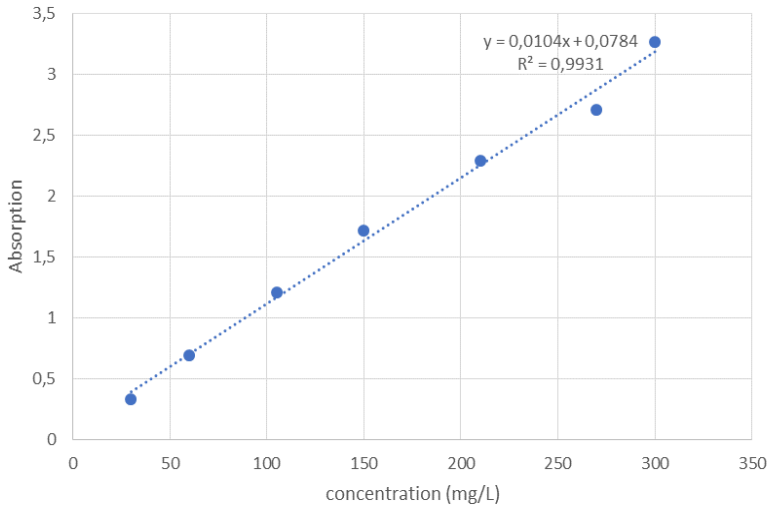

Figure 2. Gallic Acid standard curve

Table 3: Amount of total phenol in the extracts.

Chemicals	Total phenol (mg EAG / g_{ext})	
	Maceration	soxhlet
diethyl ether	25.923	117.654
Ethyl acetate	8.135	28.807
Ethanol	155.864	82.846
Water	89.673	193.038

3.5. Determination of total flavonoids:

The determination of the flavonoids was carried out by the method of $AlCl_3$ (see Figure 3 and Table 4). The flavonoid content is expressed in milligrams of quercetin equivalent per gram of extract. The amount of flavonoids was determined from the standard solution quercetin curve. The results of the assay for the two extraction techniques are grouped in Table 4.

3.6. Determination of total flavonols:

The results of the assay for the two extraction techniques are grouped in Table 5. Biochemicals are considered secondary metabolites components, the latter is directly related to antioxidant, antimicrobial, antifungal, and anticancer activities [29]. The phytochemical screening of different extracts shows the presence of phenols, flavonoids, tannins, steroids, alkaloids, saponins, quinone, and cardiac glycosides. This qualitative study revealed a near-similarity to what is found in a previous study [10]. The extraction of these products is an essential step for the valorization of plants given its dependence on the solvent and the extraction procedure. Indeed, according to the results of Table 3, for the extraction by the soxhlet method, the aqueous extract contained the highest amount (193.650 mg EAG/g_{ext}) of phenolic compounds, followed by the order ethanol (83.744 mg EAG/g_{ext}) > ethyl acetate

(29.846 mg EAG/g_{ext}), while for the extraction, the ethanolic extract contained the highest amount (155.864 mg EAG/g_{ext}) in phenolic compounds, followed by the aqueous extract (90.553 mg EAG/g_{ext}), ether extract (26.969 mg EAG/g_{ext}), and finally ethyl acetate extracted (9.227 mg EAG/g_{ext}). In this study, the flavonoids were efficiently extracted by maceration for ether, ethanol, and water, except that for ethyl acetate the soxhlet did extract a large amount. According to the results in Table 4, by maceration, the diethyl ether extracted the largest quantity (234.760 mg EAG/g_{ext}) followed by the order ethanol (170.474 mg EAG/g_{ext}) > ethyl acetate (92.429 mg EAG/g_{ext}) > water (69.496 mg EAG/g_{ext}), while through the soxhlet, ethyl acetate extracted the large amount (105.286 mg EQ /g_{ext}) followed by the order water (34.534 EAG/g_{ext}) > ethanol (13.030 mg EAG/g_{ext}). Concerning flavonols, always the maceration method is the best to extract a large quantity. With this method, ethanol extracted an amount of (188.444 mg EQ/g_{ext}) followed by diethyl ether (79.872 mg EQ/g_{ext}) then water (29.872 mg EQ/g_{ext}), and finally ethyl acetate (20.850 mg EQ/g_{ext}). For the extract by soxhlet, ethanol extracted an amount (73.481 mg EQ/g_{ext}) followed by water and ethyl acetate which extracted the same amount (~20 mg EQ/g_{ext}) followed finally by diethyl ether.

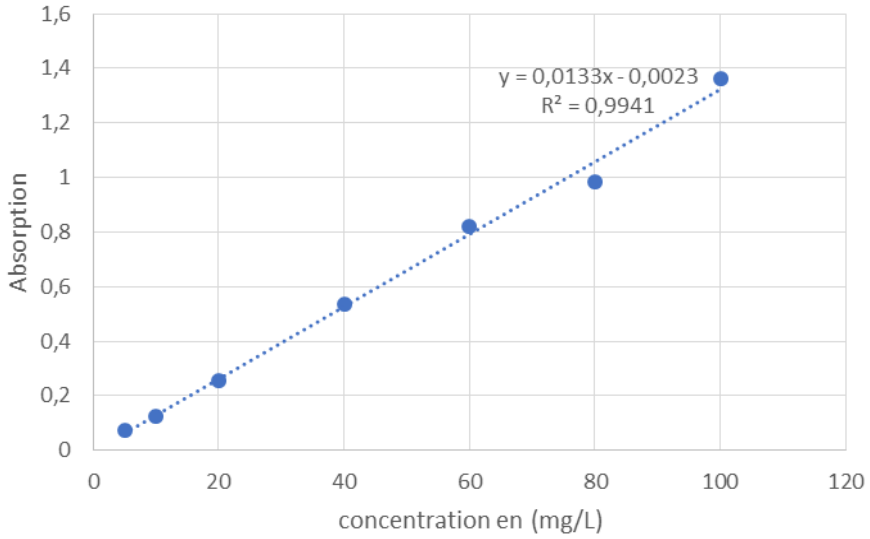

Figure 3. Quercetine standard curve

Table 4: Amount of flavonoids in the extracts.

	Maceration	soxhlet
diethyl ether	234,760	35,586
Ethyl acetate	92,429	105,286
Ethanol	170,474	13,030
Water	69,496	34,534

Table 5: Amount of flavonols in the extracts.

Chemicals	Flavonols	
	Maceration	soxhlet
diethyl ether	79.872	13.782
Ethyl acetate	20.850	20.098
Ethanol	118.444	73.481
Water	29.872	20.850

5. Conclusion

Water content, extraction yield, and phytochemical screening were determined in this work.

The results obtained showed that the humidity rate corresponds to about 50% for chamaerops humilis L. from our region. Phytochemical screening indicates the presence of phenols, flavonoids, tannins, saponins, and quinones. The results of the assay of phenolic compounds, flavonoids, and flavonols show that the contents of the latter vary according to the method followed for their extraction and also the solvent used.

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