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Phytochemical investigation of leaves and fruits extracts of *Chamaerops humilis* L.

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Abstract
The major aim of this work is the research of the bioactif compounds isolated from the *Chamaerops humilis* L. From this perspective, phytochemical study was undertaken on this western Mediterranean plant. Phytochemical Screening based on tests of colouration and precipitation were undertaken by three solvents with different polarities such as water, ethanol and diethylether. The tests carried out on leaves and fruits show presence of tannins, flavonoids and saponins. However, less presence of steroids and essential oils was observed. The selective extraction of tannins allowed us to obtain 0.351% and 0.098% yields for the leaves and fruits respectively. Separation on column chromatography conducted to a major fraction of tannins and a major compound from defatted pricarp fruits hexanic extract.

Key words: Chamaerops humilis L., Leaves, Fruits, Phytochemical screening, Extraction.

1. Introduction
Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. According to the World Health Organization (WHO) in 2008, more than 80 % of the world's population relies on traditional medicine for their primary healthcare needs [1]. Nearly, all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability [2].
The family Arecaceae comprises of 200 genera and 3000 species [3-4]. *Chamaerops humilis* L. is a medicinal plant which belongs to the Arecaceae family. It is frequently found in the North Africa especially occidental Mediterranean area [3, 5-7]. *Chamaerops humilis* can grow up between 1 to 1.5 m in mean height. But, this plant can reach 9 to 10 m of height in the protected areas. All varieties of this plant produced a single inflorescence consisting of a spadix, surrounded by a long and slender greenish yellow spathe (Figure 1).
Traditional medical practices survey carried out in Western Algeria (Tlemcen department) and Morocco revealed that *Chamaerops humilis* L. is taken as stipe or leaf extracts for the treatment of diabetes, digestive disorders, spasm, toning and gastrointestinal disorders diseases [8-10]. Besides, it plays an important role in the Algerian ecosystems [10]. Several studies have been shown the beneficial effects of *Chamaerops humilis* against chronically diseases such as cancer, ulcer, kidney stones [11-17]. Moreover, Farah Gaamoussi et al. showed that An aqueous concoction made from the leaves of *Chamaerops humilis* (L.) (dwarf fan palm), is used in the Moroccan traditional medicine for the treatment of diabetes, as well as a number of other diseases.

The results of this study validate the traditional use of the leaves of *C. humilis* in the treatment of diabetes in Morocco. Since, the aqueous leaf extract also decreased total cholesterol and triglycerides, the plant may also be useful in the management of secondary complications of diabetes (dyslipidemia) [8]. It has been reported also, that volatile compounds such as (VOCs) produced either by flowers and leaves, frequently play important roles in plant–insect interaction, and can be to attract pollinators or by to deter herbivores [18].
Nevertheless, there is insufficient information regarding the phytochemical study of *Chamaerops humilis* L such as phytochemical screening in order to detect all secondary metabolites in the parts plant; selective extraction of tannins and attempts to separate the major constituent of tannins which may be the active compound against stomach diseases.
The aim of this study was to validate the ethnomedicinal use and subsequently the isolation and characterization of the chemical constituents of *Chamaerops humilis* leaf and fruit which will be added to the potential lists of drugs.

2. Materials and Methods

Plant Material

The leaves and fruits pericarp of the *Chamaerops humilis* plant were collected from mountains located at western Algeria (Tlemcen area) in September 2010. From a geographical point of view, the districts are located, respectively in the mountains of Trara (3 districts: Djebala, Fillaoucene, Ronaine) and in the mounts of Tlemcen (2 districts: Oued Chouly (currently Oued Lakhdar) and Azails) [10]. The plant sample was identified by the authors. The voucher specimen was deposited in the Biological Science laboratory of the ecology, Department of Biology - University Abou Bakr Belkaid of Tlemcen.
The plant organs were cut into small pieces and shade dried at room temperature (20°C) for two weeks, finely powdered plant materials were stored in airtight polythene bags protected from sunlight until use.

-a- -b- -c-

Figure 1: *Chamaerops humilis* L. from mountains of Tlemcen (a: whole plant, b: leaves, c: fruits)

Extraction

Using the protocol of Nemlin and Brunel [19], 20 g each of the powdered leaves, pericarp fruits of the plant were macerated three times with 60 mL of diethyl ether for ten minutes. The extracts were filtered using Whatman filter paper and concentrated to 25 mL. The filtrates were labelled appropriately as diethyl ether extract. The marc of each part was then macerated in methanol using the same above protocol. The obtained extracts were labelled as methanol extract.
Another 5 g of each plant material was extracted by infusion in 50 mL of distilled water. After shaking for 15 minutes, the extracts were filtered through Whatman's filter paper and labelled as methanol extract.

Phytochemical screening

The phytochemical analysis was carried out respectively on the three obtained extracts diethyl ether extract, methanol extract and water extract using standard procedures to identify the constituents as described by Sofowara [20], Trease and Evans [21], and Harborne [22, 23].

Test for alkaloids
Alkaloids salts: the aqueous extract of each organs of the plant (25 mL) was stirred with 15 mL of 10 % HCl on a steam bath for 30 minutes. The mixture was extracted then three times with diethyl ether. 1 mL of the aqueous layer was treated with two drops of Wagner's reagent. Formation of brownish precipitate was regarded as evidence for the presence of salts alkaloids in the extract.
Free Alkaloids: 10 mL of organic layer (diethyl ether) was evaporated to dryness. The residue was then dissolved in 1.5 mL of HCl 2 % and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract [23] and all results were compared with blanks.

Test for flavonoids:
A small piece of magnesium ribbon was added to methanol extract (5 mL) of the each plant parts material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones.

Test for saponins:
2 g of the powdered leaves or pericarp was introduced into a beaker containing 100 mL of distilled water; the mixture was boiled in a water bath and filtered. The filtrate was completed then to 100 mL with water. In ten test tubes were introduced the following volumes (1, 2, ... 10 mL) of the mother solution. Then the final volume was readjusted to 10 mL with distilled water. All tubes were vigorously shaken for 15 s; formation of froth indicated the presence of saponins [24].

Test for steroids:
2 ml of acetic anhydride was added to 0.5 g methanol extract of each sample with 2 mL H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids:
The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids.
Salkowski test**:** 5 ml of each extract was mixed in 2 mL of chloroform, and concentrated H_2S0_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Cardiac glycosides:
Keller-Killani test was performed to assess the presence of cardiac glycosides.
5 mL of each extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish-blue colour ring may form just gradually throughout thin layer indicated the presence of cardiac glycosides [25].

Test for tannins
i)-The water extract of the crude dry powder of each organ was treated with 2 drops of 2 % $FeCl_3$ reagent. Blue dark colour and precipitate indicated the presence of hydrolysables tannins [26].
ii)-To 5 mL of each extract (5 %) was added 5 mL of concentrated HCl. The mixture was boiled for 15 minutes and filtered hot using a filter paper and collected in a beaker. Formation of red precipitate soluble in isoamylic alcohol indicated the presence of condensed tannins.

Test for anthracenosides:
To 25 mL of methanol extract were added 15 mL of 10 % HCl. The mixture was refluxed for 30 minutes. After cooling, the mixture was extracted three times with 15 mL of diethyl ether extract. After evaporation of 8 mL of etheric layer, the residue was treated with 2 mL of hot water and some drops of 10 % NH_4OH. Appearance of red oranges colour revealed the presence of anthracenosides.

Test for coumarins:
3 ml of the diethyl ether extract was evaporated to dryness in a test tube and the residue was dissolved in hot distilled water. It was then cooled and divided into two test portions, one was the reference. To the second non reference test tube, 0.5 mL of 10 % NH_4OH was added. The occurrence of an intense/fluorescence under UV light (λ_{max}= 365 nm) is a positive test for the presence of coumarins and derivatives.
Test for anthraquinones:
0.5 g of the part plant was boiled with 10 mL of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipette into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for colour changes (delicate rose pink colour showed the presence of anthraquinones).

Test for reducing compounds:
To 1 mL of the methanol concentrate was added 2 ml of distilled water. Fehling's solutions (A and B), 1 mL each were added, followed by heating in a test tube on a water bath. A brick red precipitate denotes the presence of reducing compounds.

Test for starch:
To 1 mL of aqueous extract was added 10 mL of NaCl saturated solution. After heating, starch reagent was added a blue-purplish colour is a positive test for the presence of starch.

Test for emodols:
Adding the dry etheric extract to 25 % ammonia solution a cherish-red solution indicated the presence of emodols (aglycones of anthracenosides in oxidized form).

Preparation of defatted pericarp powder
A total of 105 g of ground pieces of pericarp fruits of *Chamaerops humilis* were put into a cartridge and placed inside a Soxhlet apparatus. Then, the solvent (hexane 350 mL) was added to the round bottom flask and the mixture boiled for 2 h into 68°C under reflux. The liquid extract was collected into a flask and then concentrated under vacuum at 55°C by using a rotary evaporator. The extractive value of the oil residue (percentage yield) was calculated [27]. In order to complete our phytochemical investigation, the hexanic extract resulting from deffated pericarp fruit has been submitted to purification by column chromatography using CH_2Cl_2 as eluent.

Preparation of the tannins extract
A total of 280 g of defatted powder of each parts (leaves or pericarp fruit) were contacted with 350 mL of acetone/water (70:30, v/v) in 500 mL capped flask with timely shaking and stirring for 4 days at ambient temperature (maceration). The obtained extract was filtered by using Whatmann filter paper and then acetone was removed from the extract by using a rotary evaporator. The aqueous extract was extracted respectively with dichloromethane (2x50 mL) and 4x50 mL with diethyl acetate. The organic layer (AcOEt) was dried with Na_2SO_4, filtered and concentrated to dryness to give crude extract of tannins as a greenish solid. Separation of its different constituents by silica gel column chromatography eluted with $CHCl_3$, $CHCl_3$/MeOH (90:10, v/v) and $CHCl_3$/MeOH (80:20, v/v) gave the major fraction of tannins as a green-yellow solid [27].

3. Results and discussion

The water, diethyl ether and methanol extracts were subjected to phytochemical screening for the presence of flavonoids, alkaloids, saponins, steroids, terpenoids, tannins, anthraquinones, coumarins, reducing sugars, fatty acids, volatile oils, emodols, starch and Cardiac glycosides according to standard procedure as described above. The results of phytochemical analysis were given in the Table 1.

Table 1. Phytochemical analysis of extracts from leaves and pericarp fruits of *Chamaerops humilis* L.

Family name	**Diethyl ether extract**		**Methanol extract**		**Water extract**	
	Leaves	**Fruits**	**Leaves**	**Fruits**	**Leaves**	**Fruits**
Gallic Tannins	+	+	+	+	+	+
Cathechol tannins	-	-	-	-	-	-
Saponins	+	-	+	-	+	-
Free alkaloids	-	-	-	-	-	-
Alkaloids salts	-	-	-	-	-	-
Flavonoids	-	-	+	+	-	-
Steroids	-	+	-	+	-	-
Terpenoids	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-
Volatile oils	+	+	-	-	-	-
Fatty acids	+	+	-	-	-	-
reducing compounds	-	-	+	+	+	+
Emodols	-	-	-	-	-	-
Anthracenosides	-	-	+	-	-	-
Anthraquinones	-	-	-	-	-	+
Cardiac glycosides	-	-	-	-	-	+
Starch	-	-	-	-	-	-

Key: - : absence; +: presence

Following the protocol described in J.Bruneton's book [29], extracts of tannins were isolated successfully. The results are presented in Table 2.

Table 2. Results of extraction procedure of tannins

Organs	Weight of vegetal material (g)	Weight of tannins extract (g)	Yields (%)
Leaves	286	1.0054	0.351
(pericarp) Fruits	286	0.138	0.098

In the next step of our work, we have separated the major fraction of tannins (m = 0.142 g as yellow solid) by using the column chromatography technique. Figure 2 shows the TLC profile of this fraction.

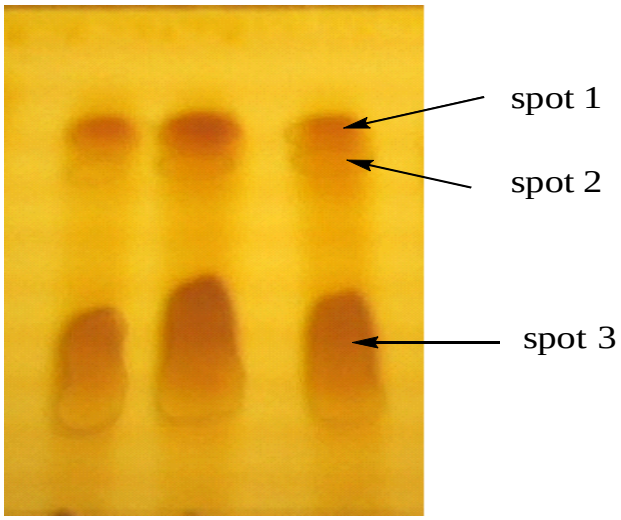

Figure 2: TLC profile of major fraction of tannins (spot 3) under iodine vapour using $CHCl_3$ /MeOH (80:20) as eluent.

In the other hand, A major product was obtained (0.6 g as brownish liquid) after column chromatography separation of hexanic extract resulting from deffated pericarp fruit. The TLC profile of this coumpound after examination under UV 254 nm and derivatization with iodine showed one spot at R_f equal to 0.46 (Figure 3).

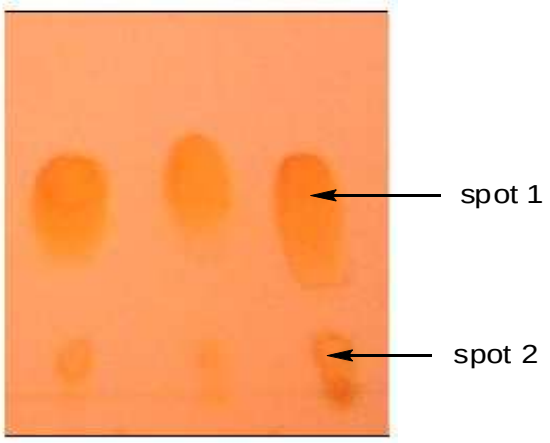

Figure 3: TLC profile of major product (spot 1) isolated from hexanic extract under iodine vapour using CH_2Cl_2 as eluant.

Analysis of the major fraction (spot 1) *via* infra red spectrum showed an intense peak at 1743.85 cm^{-1} attributed to C=O ester group. Other peaks were observed at 2923.82, 2847.34, 1459.79 and 1377.85 cm^{-1} attributed respectively to stretching and bending vibration of CH_2 and CH_3 groups. The spectrum showed also a peak at 1159.33 cm^{-1} corresponded to C-O of ester function. On the other hand, the UV spectrum revealed that this compound has as λ_{max} value 455 nm.

It is important to underline that several constituents of the leaves, stems and undergrounds parts of *Chamaerops humilis* have been investigated. The results conducted to isolate and identify many constituents such as methyl proto-dioscin and methyl proto-Pb, proto rhapissaponin from underground parts and tricin from 7-O-rutinoside from the leaves and methyl proto-dioscin and pseudo proto dioscin from the stems [28]. Besides, it has been reported that leaflets of *Chamaerops humilis* contain phenolic acids, flavonoids and tannins; after hydrolyse aglycones as quercetin and isorhamnetin were identified [29]. Basing on these literature data and ethnopharmacological results survey, a study was undertaken to isolate and separate the tannins group from the leaves and fruits of *Chamaerops humilis*.

Presence or absence of certain important compounds in an extract is determined by colour reaction of the compounds with specific chemicals reagents which acts as dyes. This procedure is a pre-requisite first step before going for detailed phytochemical investigation. The experimental results revealed the presence of gallic tannins in all extracts of leaves and pericarp fruits as indicated in Table 1. The presence of tannins is confirmed by positive reaction with ferric chloride ($FeCl_3$). Flavonoid test results showed moderately positive reaction in the presence of HCl and magnesium ribbon in
alcoholic extract. Phytochemical analysis showed the presence of saponins only in all extracts of leaves confirmed by froth test. In contrast, the study indicated that alkaloids, coumarins, starch and emodols were absent in aqueous, diethyl ether and methanol extracts of these plant parts. On the other hand, essential oils and fatty acids were weakly present in the studied organs. Qualitative phytochemical studies of reducing sugars showed a good characteristic colour and precipitate in aqueous extract using Fehling's test. Besides, anthraquinones were present in water extract, steroids in diethyl ether and methanol extracts and Cardiac glycosides in water extract of fruits part plant. Whereas, the methanol extract was found to have anthracenosides weakly only in the leaves.

From this study we observed well that the two parts plant were rich in tannins. This group of phenolic compounds have been phenolic compounds have been found to form irreversible complexes with proline rich protein [30-31] resulting in the inhibition of cell protein synthesis. Parekh and Chanda [32] reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [33]. These observations therefore support the use of *Chamaerops humilis* in herbal cure remedies.

As seen in table 2, we observed well that the leaves were richer in tannins (1.0054 g) in comparison to the pericarp fruits (0.138 g). It is important to underline that the obtained weakly yields of tannins may be due to the problem of emulsion produced in step of liquid-liquid extraction.
From figure 2, TLC of the tannins extract developed in the mobile phase of Chloroform: Methanol 80:20 and observed under UV 254 nm and after derivatization with iodine showed three spots at R_f 0.26, 0.66 and 0.72. The spot number three corresponded well to a major fraction of tannins which gave a positive reaction with $FeCl_3$.
Finally, the major product (spot 1) separated by column chromatography from the hexanic extract was analyzed using TLC two dimension which confirm the presence of one spot (figure 3). According to the spectroscopic analysis data by IR and UV, we suggested this compound isolated may be a fatty acid ester (lipid). Indeed, IR and UV analysis are only one step, we need other techniques (NMR, MS) to confirm its chemical structure.

Conclusion

Chamaerops humilis leaves- pericarp fruits, ether, methanol and aqueous extracts contains gallic tannins, steroids and terponoids, saponins and reducing sugars. From the results it is also evident that certain parts of the plant gave a positive test for a particular class of chemical compounds whereas other parts gave negative test for the same class of compounds localization of natural products. Tests for saponins, steroids, anthracenosides, anthraquinones, reducing sugars and cardiac glycosides were evident of this. The plant under investigation can be a potential source of useful drugs. However, further studies are required to isolate the pure active principal from the crude plant extracts for proper drug development. The isolation of active principle from the major fraction of tannins and structural elucidation of the pure separated constituents via modern spectroscopy techniques are in process.

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