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Exploring *Chamaerops humilis* L. fruit: physical, chemical, sensory, and FTIR analysis, along with optimization of phenolic antioxidant extraction

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ABSTRACT

The Beni Mellal-Khenifra region in Morocco boasts one of the largest forested areas in the country, rich in resources that remain underutilized. Among these resources is the *Chamaerops humilis* L. fruit, which is the focus of this study. Despite its potential value, the fruit has been largely overlooked. In this study, we comprehensively examined the physical-chemical, phytochemical screening, and sensory attributes of *Chamaerops humilis* L. fruit. Physical analysis revealed variations in fruit size, weight, providing insights into factors influencing these characteristics. Chemical analysis unveiled the fruit's moderate titratable acidity, mildly acidic pH, substantial dry matter content, and noteworthy ash content, indicative of its nutritional composition. The extraction of phenolic compounds total phenolic content TPC, total flavonoid content TFC and antioxidant DPPH (IC50) activity was optimized using simplex centroid design using various extractor solvents (acetone, water and methanol) as well as their combinations in pairs (binary) and threes (ternary). The outcomes showed that the best combination for achieving the highest levels of TPC and TFC, along with enhanced antioxidant activities was the binary acetone-water mixture. Sensory evaluation indicated a moderately acceptable taste, well-received color, and other organoleptic qualities, offering valuable insights into consumer preferences. Furthermore, Fourier transform infrared spectroscopic method with attenuated total reflectance (FTIR-ATR) spectroscopy identified seven distinctive bands in the fruit pulp spectrum, providing evidence of proteins, carbohydrates, lipids, and unique functional groups within the fruit. These findings collectively enhance our understanding of the multifaceted utility of *Chamaerops humilis* L. fruit, highlighting its potential in nutrition and traditional medicine, and emphasize the importance of further research to explore its applications and promote dietary diversity and well-being.

Keywords: *Chamaerops humilis* L., Doum; Environment, Optimization, FT-MIR spectroscopy, Simplex Centroid.

INTRODUCTION

In recent times, there has been an ever-increasing interest in exploring the potential of natural resources, particularly from forests, for their nutritional and medicinal benefits (Powell et al., 2015). These forest-derived resources offer a plethora of essential minerals, vitamins, and biologically active compounds that have captured the attention of researchers, healthcare professionals, and

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consumers alike (Ickowitz et al., 2016). Among the myriad of treasures hidden in these natural havens, wild Fruits and plants have emerged as a valuable source of sustainable nutrition and traditional medicine, garnering attention for their potential in promoting human health and well-being (Vinceti et al., 2018).

A specific focus lies on wild forest foods, including plants, which offer vital nutritional supplements, especially for rural communities, accentuating dietary diversity and overall nutritional quality in these populations (Powell et al., 2015). The increasing emphasis on sustainable diets and food systems has underscored the urgent need to preserve species diversity, particularly forest resources, worldwide. Although there are an estimated 350,000 to 450,000 known species of wild plants and animals, only a small fraction, around 5,000, have been utilized for food, and a mere 20-30 are deemed staple foods for humans (Ickowitz et al., 2014). Morocco, blessed with a diverse and rich floral wealth comprising around 4200 species and a multitude of subspecies, stands out as an essential contributor to the vast array of medicinal and aromatic plants in the Mediterranean region (Ait-Sidi-Brahim et al., 2019).

In this context, *C. humilis*, commonly known as the Mediterranean fan palm or dwarf palm, stands out as a remarkable botanical resource. Belonging to the Arecaceae family, this dioecious palm tree thrives in the western Mediterranean region, northwest Africa, and southwest Europe (Guzmán et al., 2017). Among these botanical treasures is the Moroccan wild palm tree. Names attributed to *C. humilis* vary according to the region and population (Arabic/Amazigh). Studies conducted in Morocco have documented that *C. humilis* is locally referred to as "Doum" in the Taounat region (El-Hilaly et al., 2003). In the Moroccan Middle Atlas region, encompassing Beni Mellal Khenifra, the entire plant is commonly denoted as "Doum," while the fruit is known as "Alghaz" (Derraji et al., 2023), and the term "Jmakh" is used for the palm heart (Lachkar et al., 2022). Likewise, in the Sidi Bennour region, the plant is colloquially referred to as "Eddoum," with the fruit being called "Lghaz" (Aboukhalaf et al., 2022). In Algeria, the nomenclature closely mirrors that of Morocco, where it is recognized as "Doum" (Medjati et al., 2019).

The oblong drupes of *C. humilis*, measuring between 2 to 5 cm, undergo a striking transformation as they mature, turning a distinctive red-brown hue between the months of September and November. Possessing fibrous and mildly sweet pulp, the "Doum" fruits are arranged in inflorescences or branches, each typically carrying an average of 37-91 fruits (Medjati et al., 2019).

Ethnobotanical research has shed light on the multifaceted utility of *C. humilis*, highlighting its significance as a vital source of nutritional energy (Nehdi et al., 2014). Furthermore, in traditional medicine, this palm species has been acknowledged for its diverse pharmacological properties, encompassing anti-inflammatory, anabolic, antiseptic, urinary, antilithic, and diuretic effects (Taïbi et al., 2021). Notably, the leaves of *C. humilis* have garnered attention for their antioxidative capabilities and their potential to inhibit lipoxygenase, further bolstering its standing in traditional medicine (Miguel et al., 2013). A comprehensive survey conducted in the Beni Mellal Khenifra region of Morocco unveiled the plant's application in the treatment of gastrointestinal ailments, prostate conditions, and urinary infections, all without reported adverse effects (Derraji et al., 2023). Additionally, a separate study conducted in Algeria, focusing on *C. humilis* variety argentea André, although lacking pharmacological assessments, attested to the traditional uses cited by respondents (Chetoui et al., 2021). These studies collectively underline the plant's role in phytotherapy, with applications spanning the management of pyelonephritis, prostatitis, type 2 diabetes, digestive system disorders, and veterinary medicine. Notably, the fruits are harnessed for their antidiarrheal properties and broader utility in addressing digestive system ailments (Taïbi et al., 2021).

Mixture design is a statistical technique used to study and optimize a system where the components are mixed together to form a composite (Fadil et al., 2018), widely utilized in various fields, including chemistry, pharmaceuticals, food science, and manufacturing (Alcantara et al., 2019), in our case we applied it to the extraction of phenolic compounds, particularly from *C. humilis* fruit with enhanced antioxidant activity, to our knowledge this work is the first to employ an optimization approach in extracting antioxidant compounds from *C. humilis* fruit. Solvent selection, including acetone, methanol, and water, is systematically explored through a mixture design, such as simplex centroid design efficiently identifies the optimal combination of solvents (Orives et al., 2014), focusing on achieving maximum Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity (DPPH IC50).

The objective of this study was to comprehensively investigate the physical, chemical, and sensory characteristics of *C. humilis* fruit, focusing on appearance, texture, taste, and overall sensory attributes. Additionally, the study aimed to optimize phenolic compound extraction from *C. humilis* fruit using a mixture design approach, specifically centroid simplex design, to identify the optimal solvent combination for maximum total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH IC50). Furthermore, FTIR spectroscopy was utilized to explore the chemical composition of *C. humilis* fruit, providing insight into its unique chemical components. These objectives collectively aimed to enhance understanding of the multifaceted utility of *C. humilis* fruit and promote its valorization in various applications, emphasizing the importance of sustainable resource management.

MATERIALS AND METHODS

Study area

The research was conducted in the Beni Mellal-Khenifra region (Figure 1), situated in the north-central part of Morocco, with geographical coordinates ranging from approximately 31◦ 33′ to 33◦ 46′ in north latitude and 5◦ 28′ to 7◦ 00′ in west longitude. This region spans an estimated land area of 28,088 square kilometers and is subdivided into five provinces: Beni Mellal (4528 Km^2), Khénifra (6713 Km^2), Azilal (10050 Km^2), Fquih Ben Salah (2547 Km^2), and Khouribga (4250 Km^2) (High Commission for Planning (HCP)).

The study area exhibits a diverse topography, with elevations ranging from 900 meters to 3890 meters in the mountainous regions, which comprise approximately half of the area. The other half consists of plains and plateaus at an elevation of around 600 meters (Ouatiki et al., 2019). Climate conditions within the region are highly variable, transitioning from a humid climate in the elevated mountainous zones to a semi-arid climate in the plains, characterized by harshly cold winters and scorching summers. In 2020, the region's average annual rainfall was recorded at less than 400 mm, according to the General Directorate of Meteorology in Morocco. The temperatures also exhibit significant fluctuations, with average annual temperatures varying from a peak of 46°C in August to a low of -2°C in January (General Directorate of Meteorology Morocco (GDM)). Beni Mellal-Khenifra is a significant agricultural hub in Morocco, boasting 960,000 hectares of arable land, which constitute 34% of the region's total land area. This includes 205,000 hectares of irrigated land, accounting for 21% of the region's useful agricultural area and 15% of Morocco's total irrigated agricultural land (Regional Directorate of Agriculture (RDA)).

Plant material

C. humilis fruits were collected in Beni mellal-khenifra region, located in Middle Atlas of Morocco in October 2020. The fruit was randomly picked up at their physiological maturity, transported in well-closed boxes, and stored in a dark and dry place, at the Engineering and Applied Technologies Laboratory.

Physical properties

The geometric dimensions of *C. humilis* fruit were determined. Width, and length were expressed in millimeters (mm), and Fruit weight (g) was determined using digital balance (±0.01 g sensitivity).

Chemical analysis

Determination of dry matter (DM%)

The dry matter content was determined by drying 2 g of pulp in a WEISEN isothermal oven at a temperature of 105°C. and at atmospheric pressure, until a constant mass of the sample was obtained (Audigié et al., 1980).

Determination of pH

The pH measurements were obtained following the methodology outlined by (Dowson and Aten, 1963). To initiate the process, four grams of date pulp, meticulously cut into small fragments, were dispersed in a 200 ml volume of boiling water. Following the cooling of the solution, a thermo-scientific pH-meter was employed to assess the pH value.

Determination of titratable acidity (NF V05-101, 1974)

It consists of titrating the acidity of an aqueous solution with a 0.1 N sodium hydroxide solution in the presence of phenophthalein as a colored indicator. The titratable acidity is expressed in grams of acetic acid per 100 g of products.

Determination of ash content (NF V05-113, 1972)

The dosage of the ashes is based on the destruction of all the organic matter under the effect of high temperature (500°C). Empty incineration capsules are weighed. 1 g of dry matter are added and the mass of the whole is noted. The samples are then subjected to a temperature of 550°C in a MERAEUS type MR170 muffle furnace overnight. After cooling in a desiccator, the capsules containing the ashes are weighed again. The assay is performed in triplicate for each sample.

Experimental design

The simplex centroid design was used to optimize extraction of phenolic compounds TPC, TFC, and the antioxidant DPPH IC50 activity was performed by JMP Pro 16. This statistical method is employed to systematically explore and identify the ideal combination of solvents for the extraction process, focusing on achieving maximum Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity (DPPH IC50) (Orives et al., 2014; Fadil et al., 2022).

Three different solvents acetone, methanol, and water were used in varying proportions, ranging from 0% to 100%, as shown in (Table 1). The simplex centroid design, shown in (Figure 2), included three factors: acetone, methanol and water. This plan produced nine distinct formulations, including three with pure solvents, three with binary mixtures and three with ternary mixtures.

Extract Preparation

The extraction process involved blending 1 g of *C. humilis* fruit powder with 10 ml of solvent, as outlined in (Table 1). The mixture went through vortexing for 5 minutes and sonication for 45 minutes in an ultrasonic bath situated in a dark, cool environment. Following this, the solution was centrifugation for 10 minutes at 5,000 rpm. This extraction procedure was repeated thrice. The resultant supernatants were evaporated and concentrated via a rotary evaporator. The resulting dry extracts were then reconstituted in 2 ml of methanol, filtered, and stored at 2°C until analysis. Each extraction step was carried out in triplicate to ensure precision and uniformity.

Determination of Total Phenolic Content

Total phenolic content (TPC) quantification was performed following the method described by (Arnous et al., 2002), with adaptations. In a glass tube, 2370 µl of distilled water, 30 µl of extract, and 450 µl of Folin-Ciocalteu reagent were combined. After vortexing, 150 µl of a sodium carbonate solution was added. The resulting mixture was then incubated in darkness for 2 hours, and absorbance was measured at 750 nm against a blank using a UV-visible spectrophotometer. TPC was determined using a calibration curve (ranging from 0.0156 to 1 mg.ml-1) and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE.g-1 E).

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined with modifications according to (Al-Farsi et al. 2008). Briefly, 400 µl of extract was mixed with 120 µl of 5% $NaNO_2$, followed by the addition of 120 µl of 10% $AlCl_3$ after 5 minutes. Subsequently, 800 µl of 1 M NaOH was added after 6 minutes. The absorbance was promptly read at 510 nm, and TFC was calculated as milligrams of quercetin equivalents (QE) per gram of extract (mg QE.g-1 E).

Assessment of Antioxidant Activities

The in vitro free radical scavenging activity was measured using the DPPH (1, 1 diphenyl 2, picrylhydrazyl) assay, as per (Sahin et al., 2004). This involved mixing 3900 µl of DPPH solution with 100 µl of our extract, followed by incubation in darkness for 2 hours.

The percentage inhibition was calculated using the formula:

%Inhibition= [(Abs control - Abs sample) / Abs control] × 100

The IC50 value, which signifies the concentration of the extract required to decrease the absorbance by 50%, was determined by plotting the percentage reduction of DPPH against the concentrations of the extracts. The results were expressed as milligrams of extract per milliliter (IC50, mg/ml).

Sensory evaluation

The sensory characteristics of *C. humilis* fruits was performed with 15 panelists and were conducted to determine the acceptability of the product, samples were evaluated using a 6-point organoleptic test for odor, taste, residual taste, color, and overall acceptance with a scale ranging from zero, indicating extreme dislike, to four, corresponding to like extremely (Larmond, 1979).

FT-MIR spectroscopic method

Infrared (IR) spectroscopy stands as a highly valuable analytical technique employed for examining the infrared spectra of a sample. Its foundation based on the principles of Fourier Transform (FT) spectroscopy, facilitate the conversion of a signal from the time domain to the frequency domain. In the context of FT-MIR (Fourier Transform Mid-Infrared) spectroscopy, an infrared beam is directed through the sample, and the measurement of light absorption across different wavelengths is meticulously executed. Following this data collection, a Fourier transform algorithm is adeptly employed to process and convert this information into a spectrum, effectively highlighting the distinctive absorption bands indicative of the compound's functional groups (Larkin, 2018).

FT-MIR spectroscopy finds extensive utility across diverse fields, encompassing chemistry, biology, pharmaceuticals, and materials science. Its applications encompass the identification, quantification, and investigation of the structural and chemical attributes of materials and compounds. This versatile technique accommodates the analysis of gases, liquids, and solids, catering to both qualitative and quantitative analysis needs. Notably, FT-MIR's superiority lies in its ability to provide high spectral resolution, making it capable of detecting even trace amounts of substances. Moreover, FT-MIR spectra can be compared easily with reference spectra, rendering it an invaluable tool for the identification of unfamiliar compounds (Socrates, 2004).

To investigate the functional groups existing within *C. humilis* fruits, FT-MIR spectroscopy was utilized. The spectral data was collected employing a Bruker Vector 22 FT-IR Spectrophotometer. A total of 10 samples were thoroughly examined, covering the entire spectrum from 4000 to 400 cm^{-1}.

Statistical analysis

All measurements were performed in triplicate, and the outcomes are depicted as mean values along with their corresponding standard deviations (mean ± SD). Statistical analysis was carried out using (Microsoft® Office, Santa Rosa, California, CA, USA).

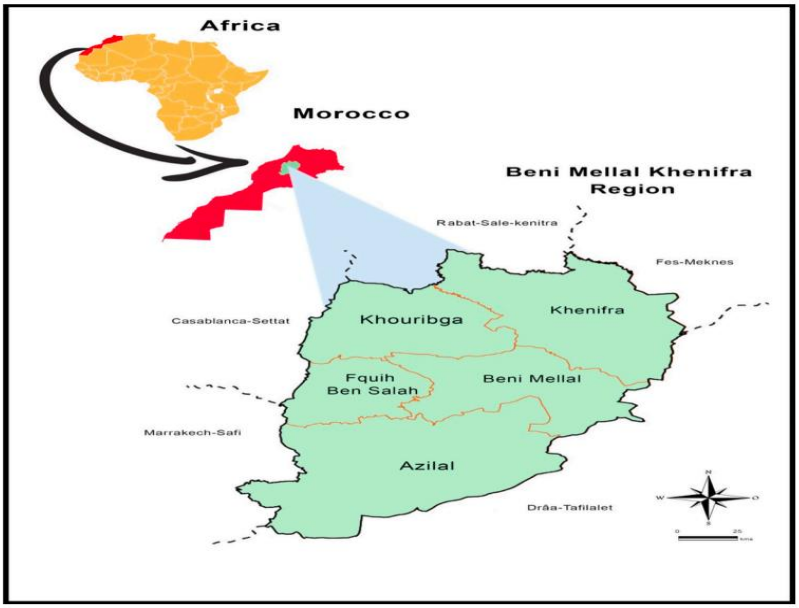

Figure 1: Geographic location of the research region.

Table 1: Proportions of Solvents in Phenolic Compound Extraction.

Runs	Water	Methanol	Acetone
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.333333	0.333333	0.333333
8	0.333333	0.333333	0.333333
9	0.333333	0.333333	0.333333

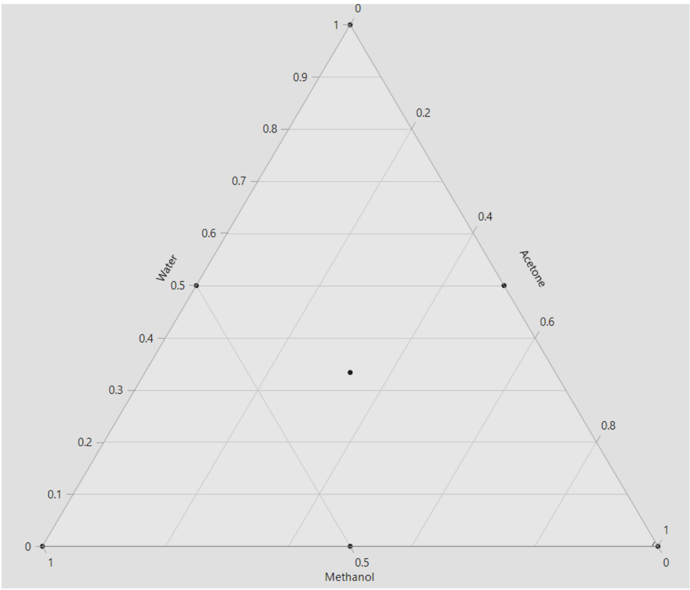

Figure 2: Simplex Centroid Design for Optimizing Extraction.

RESULTS

Physical properties

The analysis of the data (Table 2) revealed a notable variation in the dimensions of *C. humilis* fruit. The length of the fruit ranged from 15 mm to 25 mm, displaying the diverse sizes this fruit can attain. Additionally, the width varied from 16 mm to 24 mm, reflecting the range of width dimensions among the samples.

The weight of the fruit also exhibited significant variability, spanning from 2.67 g to 8.56 g. This range in weights suggests that *C. humilis* fruit can differ substantially in terms of mass. Larger fruits tended to be heavier, suggesting a positive correlation between length and weight.

Chemical analysis

Titratable Acidity (TA)

The titratable acidity of *C. humilis* fruit, recorded at 1.65±0.15, (Table 2) signifies a moderate acidity level. This characteristic significantly contributes to the fruit's overall flavor profile, impacting its taste and potential culinary applications.

pH Level

The pH level of the *C. humilis* fruit was determined to be 4.64±0.01, suggesting a mildly acidic characteristic. This pH value holds significance as it plays a role in shaping the fruit's taste, preservation duration, and adaptability for various culinary uses.

Dry Matter Content

Table 2 presents the results of our analysis, showing that the fruit of C. humilis contains 70.33% dry matter. This suggests that a large portion of the fruit is solid compared to its total weight, giving us insight into its composition and possible traits.

Ash Content

The ash content of *C. humilis* fruit, quantified at 3.93%, offers valuable insights into its mineral composition. This moderate ash content signifies the presence of minerals within the fruit, contributing significantly to its overall nutritional value.

Extraction optimization

The experimental responses obtained are displayed in (Table 3) for the simplex-centroid mixture design. This table illustrates the proportion mixture as variables, while the observed amounts of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity measured by the DPPH IC50 are presented as the responses.

The values for (TPC) and (TFC) exhibited variations among various solvent combinations, ranging from 320.06 ± 9.37 to 756.91 ± 5.56 mg GAE.g-1 E for TPC, and from 47.06 ± 0.69 to 92.45 ± 0.41 mg QE.g-1. The highest TPC value (756.91± 5.56 mg GAE.g-1 E) was observed in experiment 5, where the binary mixture of water and acetone was used as the solvent, indicating the effectiveness of this combination in extracting phenolic compounds. Similarly, the highest TFC value (92.45 ± 0.41 mg QE.g-1 E) was obtained with the binary mixture of acetone and water, suggesting that this combination is optimal for extracting flavonoids. Regarding antioxidant activity measured by the DPPH assay, the lowest IC50 value (0.345 ± 0.03 mg/ml), indicating the highest antioxidant activity, was observed in experiment 5, with the binary mixture of acetone and water. This suggests that the acetone-water mixture is most effective in scavenging DPPH radicals and exhibiting antioxidant activity.

Overall, the results suggest that the combination of acetone and water as solvent yields the highest antioxidant activity and phenolic compound content in the extract. This indicates the importance of solvent selection in optimizing the extraction process for obtaining bioactive compounds from *C. humilis* fruit.

The results of the analysis of variance (ANOVA) (Table 4) for the regression models derived from the simplex-centroid design used for solvent optimization. This table provides valuable insights into the effectiveness of the regression models in predicting the optimal solvent combination for maximizing the extraction of phenolic compounds from *C. humilis* fruit. The ANOVA results help evaluate the significance of the regression models and the individual factors contributing to the variation in the response variables, such as total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH IC50). By examining the statistical significance of the regression coefficients and the model fit, researchers can assess the robustness and reliability of the optimization process and make informed decisions regarding the selection of the most efficient solvent combination for phenolic compound extraction. Based on the ANOVA results presented in the (Table 4), the Total Phenolic Content (TPC) response shows a highly significant model with a p-value of 0.0029 and an R^2 value of 0.99, indicating that the regression model effectively explains 99% of the variability in the TPC data. Similarly, the Total Flavonoid Content (TFC) response also demonstrates a significant model with a p-value of 0.0278 and an R^2 value of 0.99, suggesting that the regression model accounts for 99% of the variability in the TFC data. The DPPH IC50 response exhibits an extremely significant model with a very low p-value of 0.0001 and an R^2 value of 0.99, indicating that the regression model excellently explains 99% of the variability in the DPPH IC50 data.

These results suggest that the regression models for all three responses (TPC, TFC, and DPPH IC50) are highly significant and effectively describe the relationship between the experimental variables. The high R^2 values further indicate that the models are suitable for predicting the responses.

Table 5 presents the results of the analysis of variance (ANOVA) for the lack of fit analysis conducted on the solvent optimization models. This analysis is essential for assessing the adequacy of the fitted models in capturing the variability of the response variables, such as total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH IC50), while also identifying any potential lack of fit. Lack of fit occurs when the regression model fails to adequately represent the underlying relationship between the independent variables (solvent combinations) and the response variables, leading to unexplained variability in the data.

After reviewing the ANOVA results (Table 5) concerning lack of fit for the responses, it was found that:

Total Phenolic Content (TPC):

The lack of fit analysis for TPC indicates a non-significant lack of fit (F = 9.16, p = 0.203), suggesting that the model adequately fits the data. The model equation represents a linear combination of the independent variables (solvents), with each coefficient indicating the effect of the corresponding solvent on the response. For instance, the coefficient for water (w), water-acetone mixture 549.12, 1289.28 respectively are suggesting that both have the highest positive effect on TPC extraction compared to other solvents in the model.

$$Y = 549.12w + 449.23m + 320.06a + 545.89wm + 1289.28wa + 306.53ma + 317.37wma$$

Total Flavonoid Content (TFC):

The lack of fit analysis for Total Flavonoid Content (TFC) mirrors that of Total Phenolic Content (TPC), displaying a non-significant lack of fit (F = 10.05, p = 0.194), implying that the model fits the data suitably. The model equation comprises a linear combination of solvents, with each coefficient reflecting the impact of the respective solvent on TFC extraction. The water-acetone mixture (wa) exhibits a notably higher coefficient of 144.22, indicating its strong influence on TFC extraction.

$$Y = 65.72w + 53.14m + 47.05a + 44.75wm + 144.22wa + 20.21ma + 51.18wma$$

DPPH IC50

The lack of fit analysis for DPPH IC50 reveals a significant lack of fit (F = 85.33, p = 0.068), indicating potential inadequacies in the model. However, the R2 value is high (0.999), suggesting that the model explains a significant portion of the variability in the data. The model equation includes both positive and negative coefficients, indicating the varying effects of solvents on DPPH IC50. For instance, the negative coefficient for wa (water-acetone interaction) exhibits a higher negative effects coefficient of -24.85 on DPPH IC50, while the positive coefficient for a (Acetone) shows the highest positive effect with a coefficient of 12.288.

$$Y= 0.831w + 2.41m + 12.288a - 3.90wm - 24.85wa - 20.79ma + 24.76wm$$

The results of the prediction profiler analysis conducted as part of the study were presented in (Figure 3). Prediction profiler analysis is a graphical tool used to visualize the relationship between multiple variables and predict the response variable's. In this context, the prediction profiler analysis aims to illustrate the impact of various solvent combinations, on the extraction of phenolic compounds from *C. humilis* fruit. According to the Prediction Profiler analysis (Figure 3), the optimal conditions for maximizing the responses TPC, TFC, and minimizing DPPH IC50 are achieved by using an acetone-water mixture with proportions of approximately 0.48 acetone and 0.52 water. Under these conditions, the predicted maximum values are 760.98 for TPC, 92.76 for TFC, and 0.13 for DPPH IC50. This suggests that the acetone-water mixture at this specific ratio is most effective in extracting phenolic compounds, resulting in higher antioxidant activity. These findings provide valuable insights into the solvent composition and proportions that yield the best outcomes in terms of phenolic extraction and antioxidant potential, highlighting the importance of solvent selection in optimizing extraction processes for bioactive compounds.

Table 6 provides a summary of the antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC) of Moroccan *C. humilis* fruits using the optimized solvent mixture composed of 52% water and 48% acetone. Shows the results for total phenolic and flavonoid content, as well as antioxidant activity, obtained from Moroccan *C. humilis* fruits using the optimized solvent mixture of 52% water and 48% acetone. The results reveal a total phenolic content (TPC) of 770±9.26 mg GAE.g-1 E and a total flavonoid content

(TFC) of 93.84±1.71 mg QE.g-1 E, both very close to the values predicted by the model. However, antioxidant activity, measured by the IC50 value, exceeded the predicted model, recording a higher value of 0.162±0.01mg/ml.

Sensory evaluation

In (Table 7) the results of the sensory evaluation conducted on *C. humilis* fruit samples. The evaluation involved 15 panelists who assessed various sensory attributes using a 6-point organoleptic test, including odor, taste, residual taste, color, and overall acceptance.

The sensory evaluation of *C. humilis* fruits, conducted by 15 panelists using a 6-point organoleptic test, revealed that these fruits exhibit a relatively mild odor (average score of 1.23±0.1) and a moderately acceptable taste (average score of 2.45±0.15). However, the aftertaste, indicated by a score of 1.02±0.09, was less preferred. On a positive note, the fruits displayed a well-received color (average score of 3.6±0.19), while the overall acceptance, with an average score of 2.53±0.25, indicated a moderate level of approval among panelists. These findings provide a comprehensive insight into the sensory characteristics of *C. humilis* fruits, highlighting their potential strengths and areas for improvement in terms of consumer acceptability and culinary applications.

FT-MIR spectroscopic method

In the FTIR-ATR spectrum (Figure 4) of *C. humilis* fruit pulp, ten distinct bands offer valuable insights into its complex chemical composition in Figure a, b. The first, a strong absorption band at 3282 cm-1, is indicative of N-H stretching vibrations within protein structures, particularly the amide A band, while also revealing O-H stretching vibrations from carbohydrates and water (Socrates, 2004). This dual presence suggests a rich blend of both proteins and carbohydrates in the fruit pulp, underscoring its biological complexity.

The band at 2921 cm^{-1}, characterized by aliphatic C-H stretching vibrations, suggests the possible presence of lipids, carbohydrates, fatty acids, cellulose, or other long-chain compounds (Socrates, 2004). Moving to the fingerprint region between 1600 and 500 cm^{-1}, several unique transmission bands emerge, offering a distinct chemical signature specific to *C. humilis* fruits. Among these, a weak band at 1663 cm^{-1} corresponds to carbonyl (C=O) stretching vibrations, hinting at the inclusion of compounds such as sugars or organic acids. Additionally, three weak bands in the spectral range of 1393-1243 cm^{-1}, although their specific molecular origins remain elusive, provide further distinguishing features of the fruit pulp. The most intense transmission band, peaking at 1032 cm^{-1}, indicates the presence of distinct chemical structures or functional groups that are notably prominent within the fruit pulp and may be linked to phosphates or other unique entities (Socrates, 2004; Max and Chapados, 2007).

Finally, six weak transmission bands spanning from 820 to 500 cm^{-1} within the fingerprint region offers intriguing insights into the molecular constituents of *C. humilis* fruit pulp. These bands may be attributed to several distinct vibrational modes. The signal at 820 cm^{-1} could possibly be assigned to skeletal C-C stretching vibrations, characteristic of the carbon backbone in monosaccharides such as glucose and fructose. Meanwhile, within this range, the bands at approximately 776 cm^{-1} might be associated with out-of-plane N-H bending vibrations, potentially corresponding to amide V. Additionally, the transmission bands in the 820-500 cm^{-1} region might also include contributions from O=C-N bending vibrations, akin to amide IV (Socrates, 2004).

In sum, these 10 bands collectively shed light on the diverse array of organic compounds, encompassing proteins, carbohydrates, lipids, and potentially unique functional groups, that contribute to the intricate chemistry of *C. humilis* L fruit pulp. Further research and comparative analysis are warranted to unveil the precise nature and proportions of these constituents.

Table 2: Physical characteristics and chemical composition of *C. humilis* fruit Results are expressed as mean ± standard deviation.

Parameter *C. humilis fruit*	**Value**
Length	19.9±2.83 mm
Width	18.95±2.11 mm
weight	4.7±1.38 g
Ph	4.64±0.01
Titratable acidity	1.65±0.15
Dry matter	70.33±1.9%
Ash content	3.93±0.08%

Table 3: Simplex-centroid mixture design experiments for extraction of antioxidant compounds and the correspondent responses.

Water	**Methanol**	**Acetone**	**TPC (mg GAE.g-1 E)**	**TFC (mg QE.g-1 E)**	**DPPH IC50 (mg/ml)**
1	0	0	549.12 ± 8.23	65.73 ± 0.45	0.831 ± 0.01
0	1	0	449.23 ± 6.15	53.15 ± 0.63	2.418 ± 0.02
0	0	1	320.06 ± 9.37	47.06 ± 0.69	12.288 ± 0.12
0.5	0.5	0	635.65 ± 7.19	70.63 ± 0.57	0.649 ± 0.04
0.5	0	0.5	756.91 ± 5.56	92.45 ± 0.41	0.345 ± 0.03
0	0.5	0.5	461.28 ± 4.36	55.16 ± 0.55	2.155 ± 0.04
0.333333	0.333333	0.333333	699.17 ± 5.81	83.73 ± 0.41	0.569 ± 0.02
0.333333	0.333333	0.333333	687.06 ± 6.16	79.71 ± 0.49	0.599 ± 0.02
0.333333	0.333333	0.333333	681.35 ± 6.59	77.92 ± 0.52	0.603 ± 0.02

Table 4: ANOVA results of regression models from simplex-centroid design for solvent optimization.

	Sum of squares	**DF**	**F value**	**P value**	**R^2**
TPC	169837.54	6	341.85	0.0029	0.99
TFC	1878.63	6	35.36	0.0278	0.99
DPPH IC50	117.26	6	56594.22	0.0001	0.99

Table 5: ANOVA Results for Lack of Fit Analysis of Solvent Optimization Models.

	Sum of squares	**DF**	**F value**	**P value**	**R^2**	**Equation**
TPC	149.30	1	9.16	0.203	0.999	Y= 549.12w + 449.23m + 320.06a + 545.89wm + 1289.28wa + 306.53ma + 317.37wma
TFC	16.10	1	10.05	0.194	0.996	Y= 65.72w + 53.14m + 47.05a + 44.75wm + 144.22wa + 20.21ma + 51.18wma
DPPH IC50	0.0007	1	85.33	0.068	0.999	Y= 0.831w + 2.41m + 12.288a – 3.90wm – 24.85wa – 20.79ma + 24.76wma

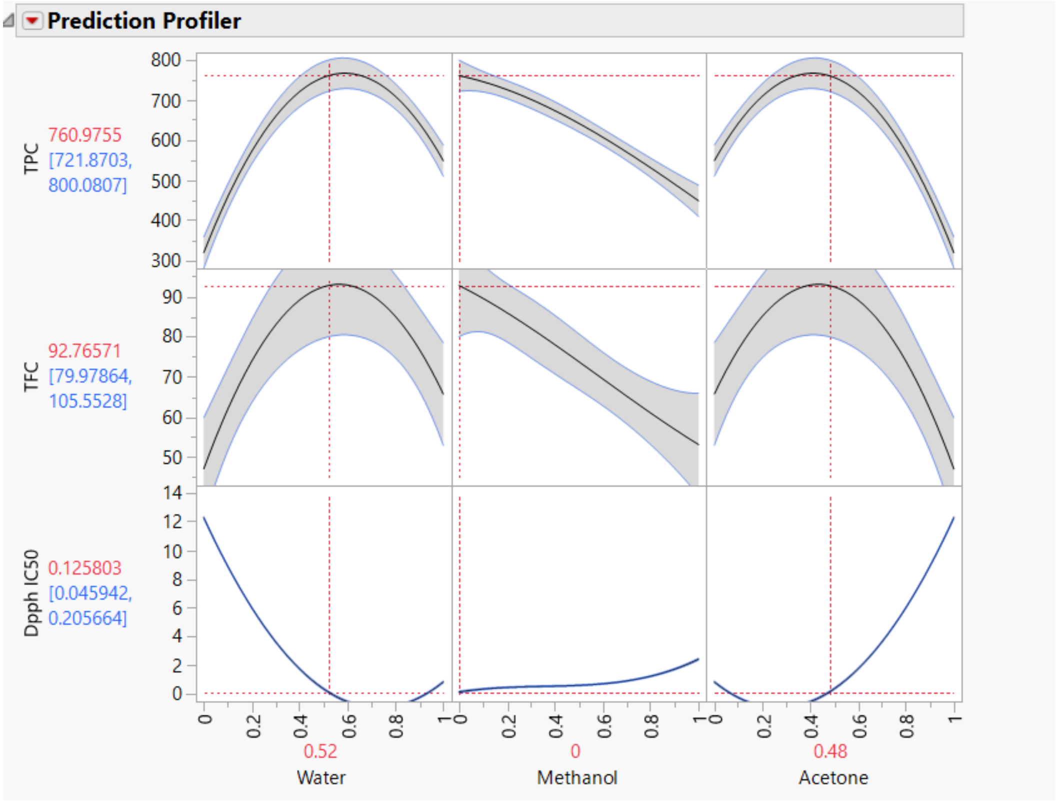

Figure 3: Prediction Profiler analysis.

Table 6: Antioxidant activity, TPC and TFC of Moroccan *C. humilis* fruits.

	TPC mg GAE.g−1 E	TFC mg QE.g−1 E	DPPH IC50 mg/ml
Chamaerops humilis L. fruits	770±9.26	93.84±1.71	0.162±0.01

Table 7: Sensory evaluation of *C. humilis* fruit samples.

Parameter	Value
Odor	1.23±0.1
Taste	2.45±0.15
Residual taste	1.02±0.09
Color	3.6±0.19
Overall acceptance	2.53±0.25

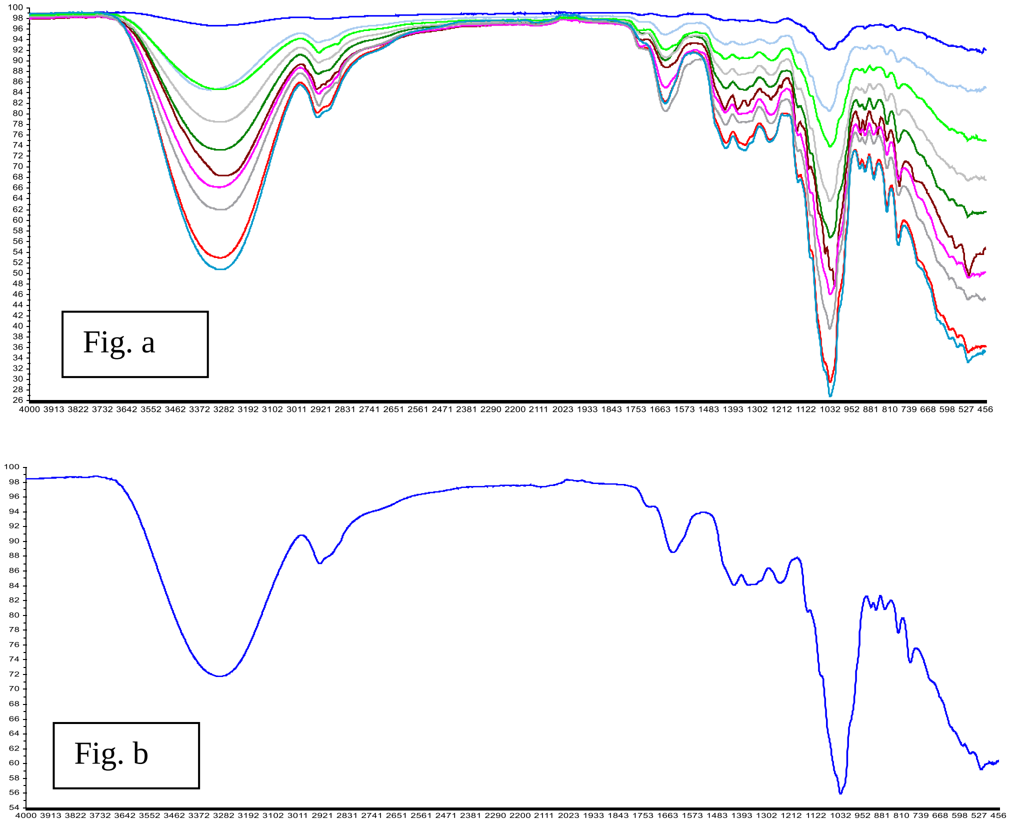

Figure 4: Characteristic Fig. a and average Fig. b FTIR-ATR spectrum of ten Chamaerops humilis L fruit pulp.

DISCUSSION

Physical properties

The significant variation in the dimensions (length and width) and weight of C. humilis fruit observed in the data can be attributed to several hypotheses related to factors that influence the physical characteristics of fruits (Ghasemi-Soloklui et al., 2023).

Genetic variation

Different genetic variations within the C. humilis species may result in varying fruit sizes and weights. Some genetic traits may favor longer or wider fruit development, while others may contribute to heavier fruits.

Environmental factors

Environmental conditions, including climate, soil composition, and local weather patterns, can have a profound impact on the growth and development of plants and their fruits. Variations in environmental conditions across different regions or even within the same region can lead to differences in fruit size and weight.

Maturity and ripeness

The stage of maturity and ripeness at which the fruits are harvested can influence their size and weight. Fruits harvested at different stages may exhibit variations in these physical characteristics.

Chemical analysis

The titratable acidity found significantly contributes to the fruit's overall flavor profile, impacting its taste and potential culinary applications. In comparison to a study conducted in Morocco, where a similar fruit displayed a slightly lower titratable acidity of 1.5, it can be inferred that *C.*

humilis fruit maintains a relatively consistent acidity level across various regions of Morocco (El Cadi et al., 2021). Conversely, a study conducted in Algeria reported a significantly lower titratable acidity of 0.23 for *C. humilis* fruit, highlighting potential regional variations in acidity levels (Bouhafsounm et al., 2019).

The pH value holds significance as it plays a role in shaping the fruit's taste, preservation duration, and adaptability for various culinary uses. When contrasting this finding with the Morocco study (El Cadi et al., 2021), where a notably more acidic pH of 3 was observed, it raises the possibility of environmental disparities or differences in fruit maturity across distinct regions, which can, in turn, influence the fruit's acidity level and flavor profile. In comparison to Algerian findings, which reported a pH of 5.0 (Bouhafsounm et al., 2019), further variations in pH levels among regions become evident.

Concerning dry matter, compared to similar studies in Morocco (El Cadi et al., 2021), which reported slightly lower dry matter content at 69.5% this emphasizes the significant presence of solid components within the fruit, excluding water. In comparison to date fruits, renowned for their high dry matter content, often exceeding 80%, *C. humilis* fruit aligns within a comparable range, signifying a noteworthy solid content.

Comparing the ash content of 3.93% to other findings from Morocco and Algeria (Bouhafsounm et al., 2019; El Cadi et al., 2021), which reported values of 3% and 5.1% respectively, highlights consistency in mineral composition across different regions. Furthermore, when juxtaposed with date fruits, renowned for their substantial mineral content spanning from 1% to 5%, *C. humilis* fruit's ash content aligns with the anticipated range, reaffirming its comparable mineral composition.

Extraction optimization

Polyphenols are natural compounds widely present in nature and are essential elements in animal nutrition. They possess numerous biological properties utilized across various industrial sectors, including antioxidant, anti-inflammatory, and antibacterial activities (Kagnou et al. 2020; Kouamé et al. 2021; Bamba et al. 2021).

When employing our optimized solvent mixture comprising 52% water and 48% acetone, *C. humilis* fruits exhibited significant levels of total phenolic content (TPC) at 770±9.26 mg GAE.g-1 E, equivalent to 92.30±1.12 mg GAE g-1 dry weight, and total flavonoid content (TFC) at 93.84±1.71 mg QE.g-1 E, equivalent to 11.26 mg QE.g-1 dry weight. These findings surpass those reported in prior studies utilizing different solvent compositions, as demonstrated by (El Cadi et al. 2021) employing ethanol-acetone and MeOH-H2O, and (Belhaoues et al. 2021) employing alternative solvent systems. This underscores the efficacy of the optimized solvent mixture in extracting bioactive compounds from *C. humilis* fruits, resulting in heightened phenolic and flavonoid content, alongside enhanced antioxidant activity, compared to alternative solvent systems used in previous research endeavors.

When testing the antioxidant activity of *C. humilis* fruits, we used a method called DPPH radical scavenging. This method is commonly used to measure antioxidant activity because it's simple, quick, and reliable. Under our optimized conditions, the IC50 of C. humilis fruits was 0.162±0.01 mg/ml. Comparing this to other studies, (El Cadi et al. 2021) found IC50 values of 1.9 ± 0.1 mg/mL and 0.4 ± 0.1 mg/mL for EtOAc and MeOH-H2O, respectively, while (Bouhafsounm et al. 2019) found IC50 values ranging from 0.76 to 3.73 mg/ml for different solvents. Clearly, our extract shows a higher ability to neutralize the radical, which may be because our fruits have higher amounts of total phenolic and flavonoids. These results match with many other studies that have shown a link between the levels of polyphenols and flavonoids in extracts and their ability to scavenge radicals (Ombouma et al. 2022).

Conclusion

The fruit of *C. humilis*, a remarkable botanical resource native to the Mediterranean region, holds promise as a source of sustainable nutrition and traditional medicine. This comprehensive study elucidated its physical attributes, chemical composition, sensory characteristics and FTIR-ATR spectroscopic profile. The fruit's diversity in size and weight reflects genetic and environmental influences, while its chemical analysis highlights its potential nutritional value, marked by moderate acidity, dry matter content and mineral composition. Sensory evaluation provides valuable insights into consumer perception. FTIR-ATR spectroscopy highlights seven distinct bands, revealing the presence of proteins, carbohydrates, lipids and unique functional groups in the fruit pulp. These results underline the multifaceted usefulness of the fruit of *C. humilis* and pave the way for further research aimed at harnessing its nutritional and medicinal potential, to promote sustainable dietary diversity and well-being. In addition, the effectiveness of the optimized solvent mixture for extracting bioactive compounds from *C. humilis* fruits, which resulted in increased phenolic and flavonoid content and enhanced antioxidant activity compared to other solvent systems used in previous research projects, suggests a promising avenue for future exploration and utilization of this valuable botanical resource.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

HD: Data collection, data analysis, and initial manuscript writing. WT: Revision and editing manuscript. FK, AO, WT: supervision.

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