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SOLVENT AND TECHNIQUES EXTRACTION INFLUENCE ON PHYTOCHEMICAL PROFILE AND FREE RADICALS INHIBITION OF *PHEONIX DACTYLIFERA* L

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ABSTRACT

The main of this investigation was to compare the extraction efficiency of different methods with various solvents from *Phoenix dactylifera* L extracts. The antioxidant activity and total phenolic compounds of extracts were evaluated using the various method. The highest extraction yield was founded in 70% methanol. Acetone extract exhibited a higher total antioxidant activity, ferric reducing antioxidant power and DPPH radical scavenging activity. The same extract also exhibited the highest phenolic, flavonoid, flavanol and condensed tannins contents. The results revealed that the ultrasonic is most suitable for the extraction of phenolic compounds and thus showing high levels of phenolic content and very high antioxidant capacities relative to the soxhlet and maceration technique. The results propose that *Phoenix dactylifera* L leaves extract can be considered as a rich source of natural bioactive compounds.

Keywords: *Phoenix dactylifera* L, Technique, Solvents, Antioxidant activity, Phenolic compounds, HPLC.

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1. INTRODUCTION

Phytochemicals, particularly antioxidants from natural sources such as fruits, vegetables, and herbs have gained popularity due to their protective properties against several chronic diseases such as cancer and cardiovascular diseases [1,2]. Antioxidants from aromatic were studied to develop natural antioxidant for food, cosmetic, and other applications [3]. The plant is rich in phenolic compounds; the type and content of polyphenols differ substantially between different parts of the plant. Many foods containing phenolic compounds, they may be known best for the one with the highest quantity. Polyphenols are spliced into family groups based on their chemical structure [4-6]. Polyphenols contained very classes: phenolic acids, flavonoids, stilbenes and lignans [7]. Extraction is an important step in the itinerary of phytochemical for obtained of bioactive constituents. Selection of a suitable extraction technique is also principal phase for the standardization of herbal products [8].

The extraction parameters presented the important step for the quality and quantity of antioxidant activity [9]. There are many techniques to recover antioxidants from plants, such as classical, Soxhlet and ultrasonic extraction, are widely used for obtaining extractive substances from *Phoenix dactylifera* L. Ultrasonic extraction has recently been shown to be very promising and effective for obtaining bioactive substances. The main benefits of the use of ultrasound are the increase of the extraction yield, a faster process and even the improved quality of the extracts [10]. However, extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent [11]. Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are aqueous mixtures containing ethanol, methanol and acetone. Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption. Methanol has been found to be more action in the extraction of lower molecular weight polyphenols, whereas aqueous acetone is better for extraction of higher molecular weight [12]. The date palm (*Phoenix dactylifera* L.) is one of mankind's oldest cultivated plants, are an Arecales species widely distributed in North Africa and Southeast Asia. *Phoenix dactylifera* L. is an evergreen tree and

can grow in the high region of course in well-drained soils [13]. This tree involves many varieties, depending on the shape and the organoleptic properties of the fruits. It is estimated that there are more than 600 varieties of this species worldwide. The aim of present work was to examine the effects of solvents and methods on the extraction of polyphenol and in vitro antioxidant activities of the extracts from *Phoenix dactylifera* L. leaves.

2. RESULTS AND DISCUSSION

Soxhlet and maceration demand long extraction time, high volume of solvents and very experience sometimes complicated [14]. Non-conventional extraction techniques such as ultrasonic-assisted extraction (UAE) have been developed, for their advantage (improved: efficiency, extract quality, extraction time or cost and volume of solvent) over conventional systems [15,16]. These methods were employed for the extraction and isolation of phenolic compounds from plants [17]. Biologically active compounds naturally existed in very small quantity in plants extracts. The composition of herbal extracts dependent on the extraction technique, the nature of phytochemicals, particle size, composition, nature of the solvent, and the presence of interfering substances [18,19]. Therefore, it is necessary to select the suitable extraction method as well as solvent based on sample matrix properties, chemical properties of the analytes, matrix analyte interaction, efficiency and desired property.

2.1. Extraction yield and phytochemical composition

Many techniques using to obtaining phytochemicals from plants such as milling, grinding, homogenization, and extraction. Among these steps, extraction is the main step for recovering and isolating phytochemicals from plant materials. Extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances [20]. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, solvent and composition of the sample are known as the most important parameters. The yields of crude extracts from the leaves of *Phoenix dactylifera* L., obtained by diverse methods using a different type of aqueous solvents, were calculated and the results were shown in Table 1. The recovery percentage of extractable compounds of the extracts is ranged from 10.66% for

aqueous acetone extract to 25.73% for aqueous methanol extract. This difference may be attributable to the higher solubility of extractable bioactive components such as carbohydrates and proteins in methanol than in ethanol and acetone [21,22]. Differences in the structure of phytochemical compounds also determine their solubility in solvents of different polarity [23]. Therefore, the addition of water to methanol proved that the extraction efficiency can be increased significantly. This observation is consistent with other reported results [24,25], as the water tends to increase the polarity of the extract. The combined use of water and organic solvent may facilitate the extraction of chemicals that are soluble in water and/or organic solvent [26].

Table 1. Extraction yield, Total phenolic, total flavonoid, total flavanol and condensed tannin contents of *Pheonix Dactylifera* L extracts.

Extract	Methanol			Ethanol			Acetone		
	Classical extraction	Ultrasound extraction	Soxhlet extraction	Classical extraction	Ultrasound extraction	Soxhlet extraction	Classical extraction	Ultrasound extraction	Soxhlet extraction
Extraction yield (%)	12.74 ± 0.05	25.73 ± 0.47	16.21 ± 0.01	12.15 ± 0.8	14.8 ± 0.54	14.95 ± 0.13	10.66 ± 0.01	15.11 ± 0.32	12.36 ± 0.12
TPC (mg GAE/g)	246.88 ± 9.73	228.29 ± 4.59	271.69 ± 1.37	182.2 ± 2.9	187.19 ± 3.84	172.64 ± 8.46	484.44 ± 4.38	528.81 ± 29.55	625.17 ± 11.82
TFC (mg RE/g)	246.19 ± 21.3	240.79 ± 20.4	269.94 ± 18.24	89.91 ± 0.1	95.53 ± 0.97	66.99 ± 0.67	205.94 ± 2.39	102.06 ± 1.90	139.8 ± 0.45
TFLC (mg QE/g)	14.16 ± 0.09	17.91 ± 0.11	10.42 ± 0.2	7.53 ± 0.3	7.70 ± 0.24	7.36 ± 0.24	26.45 ± 0.33	20.79 ± 0.6	12.14 ± 0.4
CTC (mg CE/g)	171.08 ± 9.76	195.06 ± 17.1	167.68 ± 6.63	206.05± 15.8	246.87 ± 10.23	156.68 ± 10.2	246.19 ± 21.34	240.79 ± 20.37	269.94 ± 18.24

Previously Methanol, ethanol and acetone have been commonly used as solvents at different concentrations in water for extraction of phenolic content from plant materials [27, 28].The recovery of phenolic content from the plant is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, the polarity of solvent plays a crucial role in increasing the phenolic compounds' solubility [29].

2.2. Total phenolic content

In the literature, several solvents are used for the extraction of phenolic compounds and often

mixed with water at a different proportion [30,31]. In general, phenolic compounds in plants are polar compounds, which usually are extracted with polar solvents such as aqueous acetone and methanol [32]. The ability of different solvents to extract phenolic compounds was compared using the method of Folin-Ciocalteu assay. The results were expressed as gallic acid equivalents (mg GAE/ g DW). Table 1 shows that the different results on TPP content and they were able to extract phenolic compounds, but aqueous acetone 70% was the most effective solvent as methanol and ethanol at the same concentration. Ultrasonic allows extracting the highest quantity TPP which is 625.17 ± 11.82, followed by soxhlet 528.81 ± 29.55 and ethanol classical extraction 484.44 ± 4.38 mg GAE/ g DW.

2.3. Total flavonoids content

Total flavonoid content (TFC) of different extracts was measured using aluminum chloride colorimetric methods (Table 1). The results showed that the TFC of *Phoenix dactylifera* L varied considerably from 42.93 to 205.94 mg in terms of rutin equivalents/ g DW of the sample. Flavonoids are considering as phenolic compounds with the highest antioxidant activity due to their chemical structure .Plant flavonoids are an important part of the diet because of their effect on human nutrition [9]. Even though it is apparent that the flavonoids were important phenolic compounds contributing to the antioxidant activity of date palm, it is also possible that other phenolic compounds could also contribute to the antioxidant properties of these plant [33]. In the case of both *Phoenix Dactylifera* L the highest amounts of the total phenolic compound and flavonoids are found in the extracts obtained by ultrasound extraction and classical extraction respectively, and the lowest one is obtained by the Soxhlet extraction. This may be explained by oxidation and degradation of these bioactive compounds under higher extraction temperature and the much longer extraction time of the Soxhlet extraction [34]. The plant species and the extraction method have a statistically significant influence on the total phenolic and flavonoid content in the extracts.

2.4. Total flavanol contents

Table 1 represents the total flavanol content (TFLC) of different extracts estimated using aluminum chloride colorimetric methods and expressed as quercetin equivalents (QE). The TFLC of *Phoenix Dactylifera* L extracts ranged from 7.36 to 26.45 mg of quercetin / g FW.

The classical extract prepared with 70% acetone showed highest TFLC when compared to other solvents and methods extracts.

2.5. Condensed tannin content

The analysis of the results of the content of tannins condensed consigned in table 1, reveals that the Soxhlet extraction is more effective for the extraction of the tannins (269.94 mg CE/g of fw) that the other techniques extraction (classical extraction and ultrasound extraction respectively). The increase in the temperature supports on the one hand the diffusion and the solubility of the extracted substances; on the other hand it destroys certain substance fragile [35]. This increase in the contents of tannin condensed in this extract can be explained by the destruction by the heat of the polyphenols oxidases (PPO which lower the content polyphenols; thus, the rupture of connections between polyphenols and other substances (proteins, polysaccharides...) driving with accessibility with this active ingredient can explain its share this abundance [36]. Whatever the method of extraction, acetone records the highest contents of tannins condensed (269.94 ± 18.24 mg CE / g DW) followed by ethanol and methanol are on average 246.87 ± 10,23 and 195.06 ± 17.09 Mg CE / g DW respectively. On the other hand, methanol extracts the tannins slightly. According to [37] the extraction of the tannins, in general, is carried out by a mixture of water and acetone what explains our results. [38] they are showed as acetone a 70% in water gives a better output than water or methanol.

2.6. Antioxidant activity

Large varieties of different antioxidants present in fruits, it is very difficult to measure the all the antioxidants of plant extract through a single method. Different methods have been established to measure the antioxidant capacity of different plant materials [39, 40]. Table 2 shows the antioxidative activity of *Phoenix dactylifera* L. leaves extracts in different solvents measured by different methods.

Table 2. Total antioxidant activity, IC_{50} in DPPH radical scavenging activity and Ferric reducing/antioxidant power of different extract of *Pheonix Dactylifera* L

Extract	Methanol			Ethanol			Acetone		
	Classical extraction	Ultrasound extraction	Soxhlet extraction	Classical extraction	Ultrasound extraction	Soxhlet extraction	Classical extraction	Ultrasound extraction	Soxhlet extraction
PPM	259.43 ± 1.1	268.68 ± 3.97	249.68 ± 6.12	423.44 ± 4.38	414.33 ± 12.53	432.54 ± 24.01	445.77 ± 14.43	608.34 ± 21.32	531.43 ± 16.83
DPPH IC_{50}= (µg/ml)	0,0233 ± 0.0002	0,0272 ± 0.0004	0,0268 ± 0.0001	0,0282 ± 0.0002	0,0271 ± 0.0005	0.0184 ± 0.0003	0.0144 ± 0.0002	0.0104 ± 0.0001	0.0132 ± 0.0004
FRAP	128.06 ± 3.83	136.76 ± 9.72	126.92 ± 5.51	104.29 ± 2.61	97.05 ± 5.352	116.76 ± 3.54	160.85 ± 3.71	198.94 ± 6.83	173.80 ± 9.21

2.7. Total antioxidant activity

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm [41,42]. Using this method, the result indicated that the ultrasound extract in 70% acetone of *Phoenix dactylifera* L had the highest antioxidant capacity with a value of 608.34±21.32 mg gallic acid equivalent/g fresh weight; this activity may be due to the presence of phenolic compounds [43]. The soxhlet extract in methanol showed lower activity with values of 249.68±6.12 and 445.77±14 mg gallic acid equivalent/g dried extract (Table 2). An improvement in the antioxidant capacity of extracts obtained by ultrasound was revealed by [44], which showed that the best antioxidant activities are obtained by the ultrasound method compared to soxhlet and maceration. Many of the preceding studies measured the effect of various solvents on the antioxidant activity by using different methods. [45,46] indicated that the extract of methanol showed a strong antioxidant activity measured with various methods in comparison with other solvents. However, [47] declared that the extraction in acetone gave to the most raised antioxidant activity, while methanol had the means low. In addition, [48] mentioned that the acetone extracts of the flower of lychee had an activity more raised in antioxidant analyses than of the water or methanol extracts.

2.8. DPPH radical-scavenging activity assay

The DPPH is a stable free radical and has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants [49]. It was one of the most widely used methods

for screening the antioxidant activity of vegetable materials and plant extract [50,51]. The interaction of a potent antioxidant with DPPH depends on the extraction technique and the plant species [10]. Table 2 shows the DPPH scavenging activities of the different extracts.For the plant The ultrasound extract in 70% acetone exhibited the strongest antioxidant activities against DPPH radicals IC_{50}= 0.0105 µg/ml, followed by soxhlet extract in 70% acetone with IC_{50} = 0.0115µg/ml and the lowest value in classical extract in 70% ethanol with IC_{50}= 0,0282 µg/ml.

2.9. Ferric reducing/antioxidant power assay (FRAP assay)

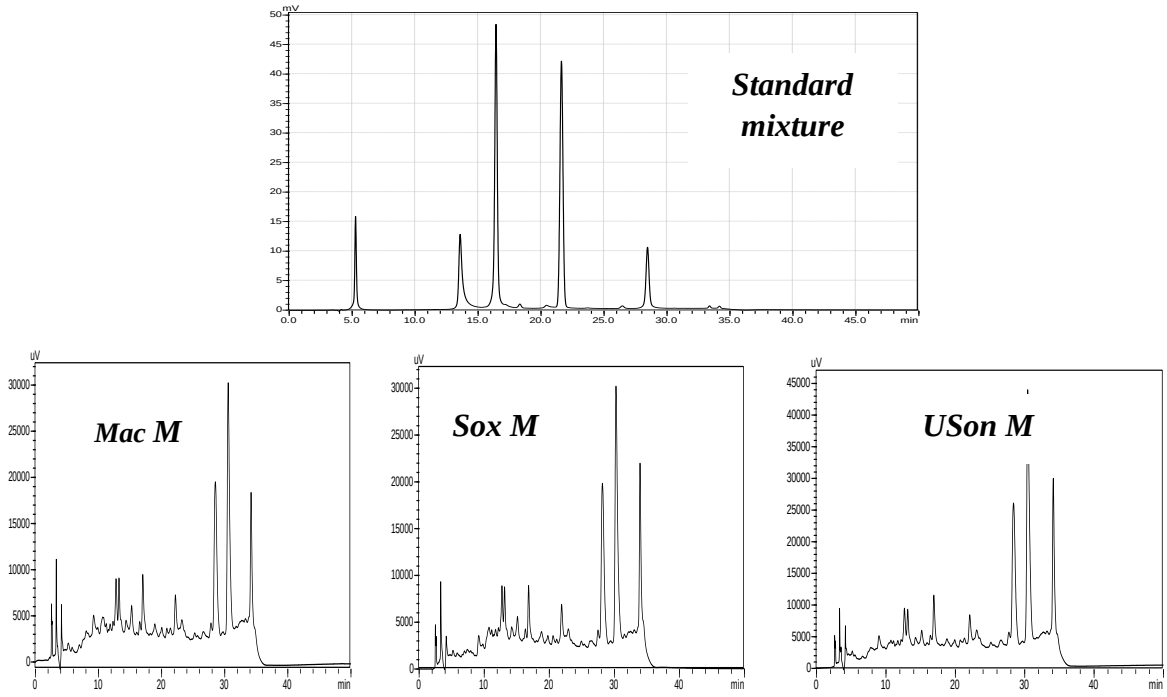
Table 2 presented the results of FRAP assay. The highest reducing power was found in ultrasound extract in 70% acetone (198.94 mg). The antioxidant activity of FRAP assay was correlated with the amount of total phenolic content. According to phenolic content ultrasound extract in 70% acetone extract showed the highest reducing activity and classical extract in 70% ethanol showed least reducing activity and they were significantly different.

2.10. HPLC analysis

HPLC fingerprinting provides the chemical characterization of the crude extract. It is known that the polyphenols and flavonoids, being secondary metabolites, are present in several plants and can serve as markers of the crude extract. Therefore, phytochemical analysis of the crude extract was assessed by analysis [52]. The constituents in the different extracts were analyzed by HPLC. Figure 1 showed the chromatograms of extracts sample and standard markers mixture. Peaks 1, 2, 3, 4 and 5 were ascorbic acid, chlorogenic acid, caffeic acid, vanillin and rutin, respectively. The contents of these components in different extracts were determined according to the calibration curves an the concentration of analyses (0–80 µg/ml). The quantitative results are summarized in Table 3. As shown, ascorbic acid was the most dominant constituent and similar in the different extracts.

Table 3. Constituents content analyzed by HPLC.

Extract	Methanol			Ethanol			Acetone		
	Classical extraction	Ultrasound extraction	Soxhlet extraction	Classical extraction	Ultrasound extraction	Soxhlet extraction	Classical extraction	Ultrasound extraction	Soxhlet extraction
Ascorbic acid (µg/mg)	0.26	0.45	0.31	0.2	0.51	0.39	190	190	200
Chloroge-nic acid (µg/mg)	0.65	1.64	0.54	1.32	1.7	1.38	1.62	1.11	0.52
Caffeic acid (µg/mg)	0.31	0.32	0.23	0.21	0.37	0.46	1.86	1.44	0.079
Vanillin (µg/mg)	0.69	0.4	0.29	0.29	0.43	0.63	0.53	0.36	0.23
Rutin (µg/mg)	6.39	8.12	6.07	7.88	11	6.2	10.4	8.37	6.44



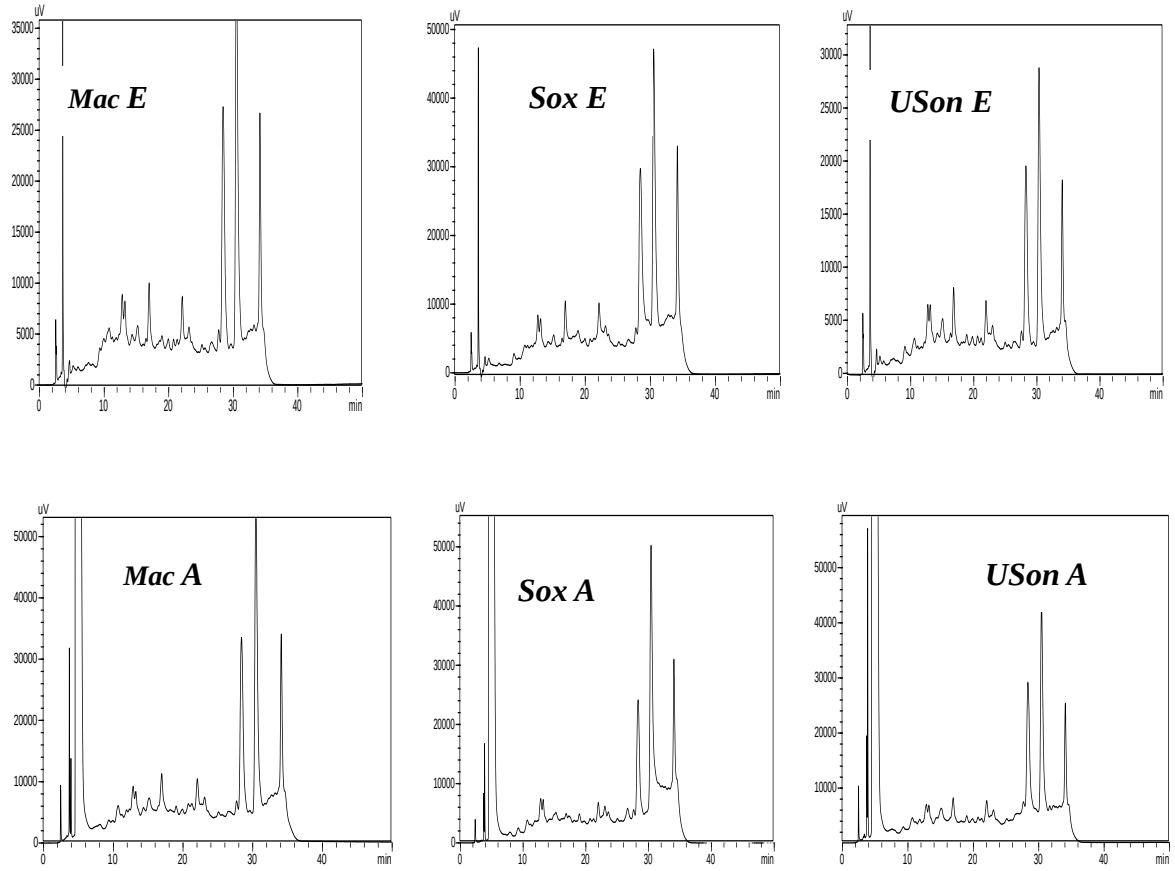


Fig.1. Chromatograms of the standard mixture and different extracts of *Phoenix Dactylifera* L leaves. Peak 1: Ascorbic acid; Peak 2: Chlorogenic acid; Peak 3: Caffeic acid; Peak 4: Vanillin; Peak 5: Rutin

3. EXPERIMENTAL

3.1. Chemical and reagents

Folin Ciocalteu (FC) reagent was purchased from PROLABO, gallic acid, rutin, quercetin, vanillin, 2, 2- diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tripyridyl-S-triazine (TPTZ) were purchased from Alpha Asear (France). Sodium carbonate anhydrous, ferric chloride, aluminium chloride, sulfuric acid and hydrochloric acid were obtained from Biochem chemopharma Co (Canada). Whereas ammonium molybdate tetrahydrate and catechin, HPLC-grade methanol, ethanol, and acetone were supplied by Sigma-Aldrich.

3.2. Plant material

The *Phoenix dactylifera* L plant used in this study were collected from southeast of Algeria, state of El Oued on December 2013. The leaves then separated from each other, washed and

dried at room temperature. These leaves were ground to a powder with a basic electric grinder and stored in the dark at room temperature before use.

3.3. Preparation of plant extracts

3.3.1. Classical extraction

15 g of powder plant material was mixed with 90 ml of 70% methanol, ethanol and acetone (1:8) separately in round bottom flasks for 24 h. Liquid extracts obtained were separated from the solid residue by vacuum filtration, concentrated using a rotary evaporator.

3.3.2. Soxhlet extraction

15 g of powder plant material was extracted successively with 70% methanol, ethanol and acetone (1:8), each 90 mL in a Soxhlet apparatus for 6 hours. All the extracts were filtered through Whatman filter paper. Then the extracts were concentrated in a rotavapor.

3.3.3. Ultrasound extraction

Ultrasonic apparatus from Ultrasons-H (40 kHz, 1500W, dimension: 15cm×50cm×14cm) was used for accelerated extraction. A beaker was partially submerged in an isothermal water bath to maintain the extraction temperature at 25 °C. Fifteen grams (15 g) leaves were then extracted with 90 ml of different solvent for 60 min. The extract was filtered under vacuum through Whatman paper, and the solvent was removed with a rotary vacuum evaporator.

3.4. Determination of polyphenol content

The total phenolic contents in each extract were determined by the Folin-Ciocalteu method [53,54]

3.5. Total flavonoids content

The total flavonoid content (TFC) of each extract was investigated using the aluminum chloride colorimetric method [55].

3.6. Total flavanol content

Total flavonol content was determined using the method of Mbaebie BO and al [56].

3.7. Condensed tannin content (CTC)

Tannin content was determined by using a method described by Thomas M [57].

3.8. Total antioxidant activity

The phosphomolybdenum method applicated for estimating the total antioxidant capacity

using the method described by Durre and Muhammad [59].

3.9. DPPH radical scavenging activity

The capacity of extracts to inhibition the DPPH radical was calculated using the method described by Rivero-Pérez et *al* [60].

3.10. The ferric reducing antioxidant power (FRAP) assay

The method described by using for determination of ferric reducing antioxidant power (FRAP) [61].

3.11. HPLC analysis

The individual component extracts were analyzed by HPLC [62]. A LC-18 column (250 mm x 4 mm i.d. x 5 mm) was employed. Samples were injected. The components of the samples were separated by gradient elution at 30 °C. The mobile phases were: A, 98:2 (v/v) acetic acid, and B. The flow rate was 0.8 ml/min and the detection wavelength was 300 nm. Phenolic compound standards Rutin, Ascorbic acid, gallic acid, Chlorogenic acid, Vanillin, Quercetin and Caffeic acid. Peak identification in HPLC analysis was realized by collation of retention time and standards reference. The mass of phenolic compounds in the extracts was done using the peak area.

3.12. Statistical analyses

The data obtained in this investigation were presented as the mean of three replicate determinations ± the standard deviation (SD).

4. CONCLUSION

The present work, presented solvents extract effect in phenolic profile, in vitro antioxidant activities of leaves extracts from *Phoenix dactylifera* L obtained by methanol, ethanol and acetone with different technique extraction. The results indicated that ultrasound extract displays high antioxidant capacity and phenolic content. The contents of flavonoids, flavanols content, and condensed tannins of the extract were decreased: acetone> ethanol>methanol. The extraction of *Phoenix dactylifera* L with different techniques revealed ultrasound to be the finest to recover the antioxidant capacity, followed by Soxhlet, and classical extraction. This research case explained the advantage of the ultrasound, compared to the conventional extraction methods both for polyphenols. These results suggest that this plant has potent

bioactive compounds and natural preservative therapeutic resources for treating various diseases.

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