Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(1); 1-7

ISSN: 0975-4873

Research Article

Optimization of Ultrasonic Extraction of Phenolic Compounds from *Phoenix dactylifera* L and Evaluation *In Vitro* of Antioxidant and Anti-inflammatory Activity

Laouini Salah Eddine[1*], Ladjel Segni[2], Ouahrani Mohammed Ridha[1]

[1]*Laboratory of Valorization and Technology of Saharian resources, El-Oued University, Algeria*
[2]*Process engineering Laboratory, Kasdi Merbah University, Ouargla, Algeria*

Article Online: 1st Feb, 2015

ABSTRACT
The present study was undertaken on optimization extraction of phenolic compounds in the leaves of Ghars variety from *Phoenix dactylifera* L by ultrasound assisted extraction (UAE) using ethanol, methanol, hexane and chloroform as the extraction solvent. This study investigated the influence of various parameters time (10, 20, 30, 40 and 50 min), temperature of extraction (30, 40, 50 and 60 °C), volume of solvent on the extraction (40, 60, 80 and 100 ml) in composition of extracts. Phenolic content, proanthocyanidins were investigated. The antioxidant properties of the extracts were analyzed by the ferric reducing antioxidant power (FRAP), superoxide radical scavenging $(O_2)^-$ and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assays. Positive effect of increase of the sonication time and/or temperature on the scavenging activity of the extracts against free DPPH radicals and thus lower IC_{50} values was demonstrated. Strong linear correlation of DPPH radical scavenging capacities of the extracts with content of phenolic was established. FRAP values significantly correlated with total proanthocyanidins content in the extracts. the extracts of 20 min was presented the high antioxidant and anti-inflammatory for or time and the volume of 80 ml give the optimum fraction between the powder plant and solvent. From an orthogonal design test, the best combination of parameters was 80 ml of ethanol as extraction volume, 20 min of extraction time and 60 °C of ultrasonic temperature. It was concluded that ultrasonic extracts of Ghars variety from *Phoenix dactylifera* L of hold considerable potential against free radical toxicity by virtue of their polyphenolic constituents, and might have significant clinical roles in prospect.
Keywords*: Phoenix dactylifera* L; optimization; ultrasonic extraction; phenolic content antioxidant activity.

INTRODUCTION

Over the past years great attention has been paid to plant phenolic compounds. These secondary plant metabolites, naturally present in all organs of plants, are part of our everyday diet. Phenolic compounds are plant secondary metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants[1]. Phenolic com-pounds have a variety of physiological activity, such as antioxidant, antimutagenic, antiallergenic, anti-inflammatory, antimicrobial, anticoagulant, antipeptic antiproliferative, antitumoral, antibacterial, antiviral, and antiadhesive activities[2,34,5] and play a role in preventing human neurodegenerative diseases, cardiovascular disorders and cancer[6]. This compounds are now widely used in the fields of biology, medicine, food, and so on. Reactive oxygen species (ROS) is generated in living organisms during metabolism[7]. It is produced in the forms of superoxide anion $(O_2)^-$ hydroxyl radical ($OH^\cdot$), hydrogen peroxide (H_2O_2)and nitric oxide (NO). Excessive amounts of ROS may be harmful because they can initiate biomolecular oxidations which lead to cell injury and death, and create oxidative stress which results in numerous diseases and disorders such as cancer, stroke, myocardial infarction, diabetes, septic and hemorrhagic shock, Alzheimer's and Parkinson's diseases. In addition, oxidative stress may cause inadvertent enzyme activation and oxidative damage to cellular systems[8]. The negative effects of oxidative stress may be mitigated by antioxidants.

Natural antioxidants are assumed to be less toxic than synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT), which are suspected of being carcinogenic and causing liver damage[9,10]. It is believed that an increased intake of food, which is rich in natural antioxidants is associated with a lower risk of degenerative diseases, particularly cardiovascular diseases and cancer[11].

Phoenix dactylifera L. (synonyms Palma major Garsault and Phoenix cycadifolia Hort. Attens ex Regel) commonly known as the date palm is an important plant in the scorched regions of Southwest Asia and North Africa, are used to treat a variety of ailments in the various traditional systems of medicine[12,13]. A literature search did not yield any reference about earlier reports on the ultrasonic leaves extract of phenolic compounds from Ghars variety from *Phoenix dactylifera* L. In the present study, ultrasound-assisted extraction for the polyphenols enriched extract of Ghars variety from *Phoenix*

**Author for Correspondence*

Table 1: Effect of solvent volume on the antioxidant activity of ethanolic leaves extracts of Ghars variety from Phoenix Dactylifera L at 60 °C and 20 min

Solvent volume (ml)	DPPH·	FRAP	O_2^-	NO
20	23.19±0.86	29.55±1.12	4.78±0.03	48.72±1.51
40	28.72±0.82	35.69±1.07	8.88±0.05	55.29±1.72
60	34.69±0.94	42.31±1.26	14.57±0.52	63.56±1.85
80	41.43±1.52	56.74±1.97	22.12±0.54	71.45±2.07
100	40.87±1.39	54.28±1.92	20.33±0.61	67.33±1.98
120	40.38±1.13	52.73±1.67	19.98±0.71	64.12±1.93

Values are mean ± standard deviation of three separate determinations (n=3). All results are significantly at p<0.05. DPPH, % inhibition of DPPH· at concentration of 10 µg/ml. O_2^-% inhibition of O_2^-at concentration of 10 µg/ml. FRAP, expressed as mg/ml of Fe(II) at concentration of 500 µg/ml. NO, % inhibition of NO at concentration of 500 µg/ml.

Table 2: Antioxidant activity of ethanolic, ethyl acetate, chloroform and hexane leaves extracts of Ghars variety from Phoenix Dactylifera L at 20 min, 60 °C and solid–solvent ratio 1:8

Extracts	IC_{50}			
	DPPH·	FRAP	O_2^-	NO
Ethanolic	13.57±0.54	442.38±9.32	64.79±1.81	375.41±8.51
Methanolic	19.22±0.54	478.59±10.37	69.31±1.53	392.44±10.64
Chloroform	26.88±0.91	508.75±10.18	78.92±1.75	424.12±12.45
Hexane	38.26±1.15	529.11±14.06	91.71±2.14	451.66±13.88

Values are mean ± standard deviation of three separate determinations (n=3). All results are significantly at p<0.05. IC_{50} values were expressed as µg/ml.

dactylifera L were investigated and the operational parameters were optimized. The ultrasound-assisted extraction for the polyphenols was also compared with other methods. Furthermore, the antioxidant and anti-inflammatory activity of polyphenols Ghars variety from *Phoenix dactylifera* L was also determined in multitest systems in vitro. The objective of the work is to establish the optimized condition of ultrasound assisted extraction for the polyphenols enriched extract from F. eucommiae and provide bioactivity information about flavonoids of Ghars variety from *Phoenix dactylifera* L for development and application of the resource.

MATERIALS AND METHODS

Plant material and extraction: The leaves of selected variety (Ghars) from *Phoenix Dactylifera* L were collected from southeast of Algeria, state of El Oued on December 2012. After, were thoroughly washed and reduced into small pieces before being ground and powdered into particles (about 1 mm in size). Then the powder was put in a hot air oven at 60 °C until complete drying. Depending on the physical characteristics of the samples, the time ranged from 18 at 30 h. Ultrasonic apparatus from Branson (40 kHz, 1500W, dimension: 49 cm×14 cm×15 cm) was used for accelerated extraction. A beaker was partially submerged in an isothermal water bath to maintain the extraction in selected temperature. 10 g were then extracted with ethanol, ethyl acetate, hexane and chloroform for 10, 20, 30, 40 and 50 min. The extract was filtered through Whatman No. 4 paper on a Büchner funnel by vacuum; the solids were washed with an additional 50 ml for each solvent. The filtrate was rotary-evaporated under vacuum at 40 ◦C to dryness. The crude extracts were dried in a vacuum oven at 45 ◦C. Extracts were stored at +4 °C in dark until use.

Determination of total phenolic content: The total phenolic contents in the selected variety was determined by the folin-Ciocalteus method[14,15]. Briefly, 100 µL of both the sample and the standard (gallic acid) of known

Table 3: Effect of extraction time on the antioxidant activity of ethanolic leaves extracts of Ghars variety from Phoenix Dactylifera L at 60 °C and solid–solvent ratio 1:8

Time (min)	DPPH·	FRAP	O_2^-	NO
10	32.59±1.06	44.35±1.72	15.29±0.63	63.55±1.73
20	41.43±1.52	56.74±1.97	22.12±0.54	71.45±2.07
30	41.09±1.39	55.32±1.59	21.17±0.66	70.85±1.86
40	40.59±1.47	54.91±1.72	20.95±0.57	69.89±2.02
50	40.22±1.55	54.19±1.61	20.25±1.55	68.83±2.08
60	39.77±1.22	53.35±1.69	20.11±0.68	68.07±1.82

Values are mean ± standard deviation of three separate determinations (n=3). All results are significantly at p<0.05. DPPH, % inhibition of DPPH· at concentration of 10 µg/ml. O_2^-, % inhibition of O_2^- at concentration of 10 µg/ml. FRAP, expressed as mg/ml of Fe(II) at concentration of 500 µg/ml. NO, % inhibition of NO at concentration of 500 µg/ml.

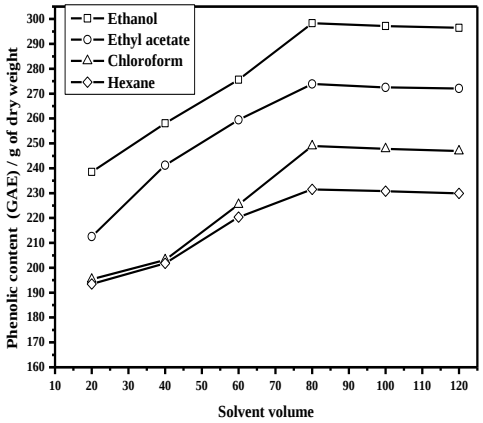

Fig 1: Effect of solvent volume on the kinetics of ethanol, ethanol, ethyl acetate, chloroform and hexane leaves extracts of Ghars variety from Phoenix Dactylifera L at 60 °C and 20 min.

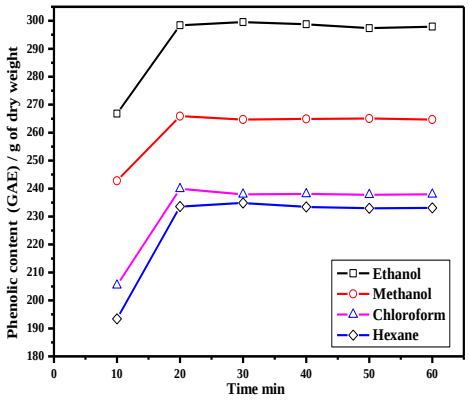

Fig. 2: Time effect on the kinetics of ethanol, ethanol, ethyl acetate, chloroform and hexane leaves extracts of Ghars variety from Phoenix Dactylifera L at solid–solvent ratio 1:8 and 60 °C.

concentrations were made up to 2.5 ml with water and mixed with 0.25 ml of 1N Folin-ciocalteus reagent. After 5 min, 2.5 ml of sodium carbonate aqueous solution (2%, w/v) was added to the mixture and was completed the reaction for 30 minutes in darkness at room temperature. The absorbance was read at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). For the blank the same protocol was used but the extract was replaced by solvent. The concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight using UV-Visible (Shimadzu UV-1800, Japan) and the equation of calibration curve: Y= 0.00778x, R^2 =0.991, x was the absorbance and Y was the gallic acid equivalent. All results presented are means (±SEM) and were analyzed in three replications.

Determination of condensed tannins(proanthocyanidins): Determination of proanthocyanidins content was determined using a spectrophotometric method[16]. A volume of 0.5 ml of different extracts or standard (catechin) was added to the mixture of 3 ml of 4% vanillin- methanol (4%, v/v), 1.5 ml of hydrochloric acid and then vortexed. The resulting mixture was allowed to stand for 15 min at room temperature, the absorbance of each was measured at 500 nm using spectrophotometer (Shimadzu UV-1800, Japan). Total proanthocyanidin content was calculated as mg catechin equivalent (mg CTE/g) using the equation obtained from the calibration curve: Y = 0.5617x, R^2 = 0.985, where x is the absorbance and Y is the catechin equivalent.

DPPH radical scavenging activity: 1 ml aliquot of each extract was added to 0.5 ml of a DPPH ethanolic solution (7.8 mg DPPH in 100 ml of each extraction solvent). The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature[17]. The antioxidant activity was then measured by the decrease in absorption at 517 nm using spectrophotometer and corresponds to the extract ability to reduce the radical DPPH to the yellow-colored diphenilpicryldrazine. The antiradical activity was expressed as IC_{50} (µl/ml). the antiradical percentage inhibition calculated by the following equation:

DPPH scavenging activity = $(A_o - A_1) / A_o \times 100$.. (1)

Where A_o is the absorbance of control test after 30 min, A_1 is the absorbance of the sample extract after 30 min. All results are means (±SEM) and were analyzed in triplicate.

Measurement of ferric reducing antioxidant power (FRAP assay): The reducing power was determined by using FRAP assay[18]: Briefly, the FRAP reagent contained 2.5 ml of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3M acetate buffer

Table 4: Temperature effect of extraction on the antioxidant activity of ethanolic leaves extracts of Ghars variety from Phoenix Dactylifera L at 20 min and solid–solvent ratio 1:8

Temperature (°C)	DPPH˙	FRAP	O_2^-	NO
30	22.58±0.81	34.77±1.17	09.±0.31	45.61±1.74
40	29.88±1.08	41.08±1.21	13.07±0.51	56.11±2.03
50	35.25±1.14	48.59±1.33	16.44±0.61	64.96±2.16
60	41.43±1.52	56.74±1.97	22.12±0.54	71.45±2.07

Values are mean ± standard deviation of three separate determinations (n=3). All results are significantly at $p<0.05$. DPPH, % inhibition of DPPH˙ at concentration of 10 µg/ml. O_2^-, % inhibition of O_2^- at concentration of 10 µg/mL. FRAP, expressed as mg/ml of Fe(II) at concentration of 500 µg/ml. NO, % inhibition of NO at concentration of 500 µg/ml.

(pH 3.6), was freshly prepared. A volume 0.2 of ethanolic extract (various concentrations) or standard was mixed with 1.8 ml of freshly prepared FRAP reagent . The absorbance of each sample solution was subsequently measured at 595 nm. For the calibration curve, $FeSO_4$ was prepared in same solvent extraction in the range of 100–700 µM . The results were expressed as mg/ml of Fe(II), using the equation obtained from the calibration curve of $FeSO_4$: $Y = 6.908x$, $R^2 = 0.998$.

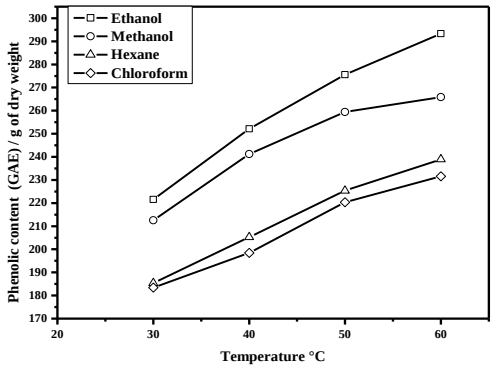

Fig. 3: Temperature effect on the kinetics of ethanol, ethyl acetate, chloroform and hexane leaves extracts of Ghars variety from Phoenix Dactylifera L at solid–solvent ratio 1:8 and 20 min.

Scavenging activity of superoxide radicals: The superoxide anion scavenging of extracts was estimated using the inhibition of NBT reduction by photochemical generated O_2^-. To the assay mixture contained 2 µM of riboflavin, we added 6 µM EDTA, 50 µM NBT and 3 µg of sodium cyanide in 67 mM phosphate buffer (pH= 7.8) in a final volume of 3 ml. Initial absorbance was measured at 530 nm, the tubes were illuminated uniformly with incandescent lamp at 530 nm. The sample extract was added to the reaction mixture, in which O_2^- radicals are scavenged, thereby inhibition the NBT reduction[19]. Quercetin used as a positive control and the percentage of scavenging inhibition was calculated using equation 1.

Nitric oxide generation and determination by Griess reagent: Nitric oxide was produced from sodium nitroprusside. It interacts with oxygen to produce nitrite ion and determined by the use of Griess reagent[20]. A volume of 2 ml of sodium nitroprusside prepared in saline phosphate buffer (pH= 7.4) was added to 0.5 ml of different concentrations of plant extracts. The mixture was set at 25 °C for 150 min. 0.5 ml of each sample from above solutions were added to 0.5 mL of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphtylethylen diamine dihydrochloride) and allowed to stand for 30 min. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphtylethylene diamine was measured at 546 nm. The amount of nitric oxide radicals was calculated using the equation 1.

Statistical analysis: All analyses were carried out in triplicates. Data were presented as mean ± standard deviation (SD). Statistical analyses were performed by one-way ANOVA. Significant differences between groups were determined at $P < 0.05$. Statistical calculations were carried out by OriginPro Version 8.0 software (OriginLab Corporation). Values of $p < 0.05$ were regarded as significant and values of $p < 0.01$ were regarded as very significant.

RESULTS

Time, solid-solvent ratio and temperature of extraction are important parameters to be optimized. Kinetics of ethanolic, methanolic, hexane and chloroform extraction of polyphenols from Ghars variety of *Phoenix dactylifera* L is presented in fig. 1, fig. 2 and fig. 3.

The selected solvent volumes used in this study were 20, 40, 60, 80, 100 and 120 ml. Other parameters were kept constant, time extraction 20 min and temperature 60 °C. The results show solvent volume had a significant effect ($p < 0.05$) on the extraction of phenolic contents (fig. 1). The best solvent volume that yielded the highest phenolic content was 80 ml, followed by 100, 120, 60, 40 and 100 ml of solvent, show that the concentration of polyphenols grows logically while the ratio increases. The phenolic content of ethanolic extracts at 20, 40, 60, 80, 100 and 120 ml were 238.54 ±6.56, 258.11±6.78, 275.57±7.12, 298.34±7.52, 297.22±7.81 and 296.43±7.73 mg AGE/ g dry weight consequently. For the extraction solvent, ethanol were much higher than the obtained with ethyl acetate, hexane and chloroform as solvent, the phenolic content of ethanolic, ethyl acetate, chloroform and hexane extracts were 298.34±7.52, 273.88±6.27, 248.93±6.31, 231.55±6.38 mg AGE/ g dry weight.

The antioxidant capacity was in accordance with the phenolic content in the extracts. The results show that % inhibition of $DPPH^{\cdot}$, FRAP and O_2^- was higher in the ultrasonication extracts for ratio 1:8 compared to the other ratio. For the ratio of 1:8, utrasonicated leaves extracts exhibited % inhibition of $DPPH^{\cdot}$, FRAP and O_2^- of 41.43±1.52% (concentration of 10 µg/ml), 56.74±1.97% (concentration of 500 µg/ml) and 22.12±0.54 (concentration of 10 µg/ml) respectively. The ration of 1:2 showed the lowest inhibition (23.19±0.86%, for DPPH at 10 µg/ml , 29.55±1.12% for FRAP at 500 µg/ml and 4.78±0.03 for O_2^- at 10 µg/ml). The antioxidant activities decreased following the same sequence: extract of ration 1:8 > extract of ration 1:10 > extract of ration 1:12> extract of ration 1:6 > extract of ration 1:4> extract of ration 1:2 (Table 1).

The same patterns of activities were revealed as in the anti-inflammatory capacity: extract of ratio 1:8 > extract of ration 1:10 > extract of ration 1:12> extract of ration 1:6 > extract of ration 1:4> extract of ration 1:2 Thus, the extract ration of 1:8 had the higher anti-inflammatory capacity (Table 1). Altogether, these results suggested that the leaves ethanolic extract of solid-solvent ration 1:8 possessed excellent antioxidant and anti-inflammatory property. For the extraction solvent, ethanolic extract inhibited radicals $DPPH^{\cdot}$, FRAP, O_2^-

and NO were much higher than the obtained with methanol, hexane and chloroform extracts (Table 2).

The effect of extraction time on extraction of phenolic compounds was study, extraction process was carried out using extraction time from 10 to 60 min, while other parameters were as following: temperature 60 °C and ratio of solid-solvent to raw material 1:8 g/ml. The effect of extraction time on extraction yield of phenolic compounds of leaves extracts of Ghars variety from *Phoenix dactylifera* L is shown in figure 2. the amount of extracted polyphenols increased continuously with the time, the variance of extraction phenolic compounds is relatively rapid and reaches a maximum at 20 min and then started reducing at the ultrasonication time of 30 min.

The phenolic content of ethanolic extracts at 10, 20, 30, 40, 50 and 60 min were 266.78 ±5.70, 298.43±7.84, 299.51±8.97, 298.74±7.85, 297.33±7.15 and 297.88±7.64 mg AGE/g dry weight consequently.

For the antioxidant activity, the results showed that an ultrasonication time of 20 min inhibited the highest radicals from leaves extract . The antioxidant capacity of leaves extracts started reducing at the ultrasonication time of 30 min, this observed and were found in $DPPH^{\cdot}$, FRAP and O_2^- values. The antioxidant activities for extracts decreased following the same sequence: extract time of 20 min > extract time of 30 min > extract time of 40 min > extract time of 50 min > extract time of 60 min> extract time of 10 min (Table 3). The same patterns of activities were revealed as in the anti-inflammatory capacity.

For the temperature effect, The selected temperatures for ultrasonication were 30, 40, 50 and 60 °C. The constant parameters consisted of 80 ml of solvent extract and 20 min of ultrasonication time. The results show that ultrasonic temperature had a significant effect ($p < 0.05$) on the phenolic contents of leaves extracts (fig. 3). The obtained results show a very clear influence of the temperature on the extraction of polyphenols from leaves of Ghars variety from *Phoenix dactylifera* L and was found to increase the phenolic content of the leaves extracts compared to phenolic extraction without heat application. It was found that the recovery of phenolic content was parallel to the increase of temperature from 30 to 60 °C. The optimum temperature that yielded the highest phenolic content was 60 °C, followed by 50, 40 and 30 °C . The phenolic content of ethanolic extracts at 30, 40, 50 and 60 °C were 221.54±5.08, 252.11±6.64, 275.57±7.12 and 293.34±78.31 mg AGE/ g dry weight consequently.

The temperature has a positive effect on the activity antioxidant of leaves extract. The obtained results show also a very clear influence of the medium temperature on the inhibition of studies radicals ($DPPH^{\cdot}$, FRAP, O_2^- and NO) by the polyphenols from Ghars variety of *Phoenix dactylifera* L (Table 4). At 60 °C the antioxidant activity were doubled comparing to the antioxidant activity obtained at 20 °C for $DPPH^{\cdot}$(concentration of 10 µg/mL) from 22.58±0.81% at 20 °C to 41.43±1.52% at 60 °C.

The antioxidant activities for extracts decreased following the sequence: extract at 60 °C > extract at 50 °C > extract at 40 °C > extract at 30 °C. (Table 4). Similar trends were found in anti-inflammatory activity , where 60 °C of ultrasonication temperature resulted in the highest inhibition of nitric oxide of leaves extract.

DISCUSSION

In the present study, ultrasound-assisted extraction was employed for phenolic extract from *Phoenix dactylifera* L. The operational parameters were optimized using ultrasonic apparatus from Branson (40 kHz, 1500W). Before the optimized experiment, the main parameters independently influencing phenolic extract from by ultrasonic-assisted extraction were investigated as extraction temperature (varying from 30 to 60 °C), ultrasonic extraction time (varying from 10 to 60 min), solid to liquid ratio (varying from 1:2 to 1:12) and different solvent (ethanol, ethyl acetate, hexane and chloroform). The antioxidant and anti-inflammatory activities of extracts at different parameters were investigated. The majority of the literature reported the influence of extraction solvent on the polyphenols extracts[21,22]. The total phenolic content in extracts solvent were as follows; ethanolic extract > ethyl acetate extract> chloroform extract> hexane extract, While ethanol is believed to disrupt the bonding between the solutes and plant matrices[23]. Moreover, The affinity of ethanol toward the solubilisation of plant materials (leaves) may be related to the dielectric constant [ethanol (24.3) > ethyl acetate (6.02) > chloroform (4.8) > hexane (2.02)][24]. Therefore, the results may be related to the solvent polarity and the solubility of polyphenols in leaves extracts of Ghars variety from *Phoenix dactylifera* L and ethanol is good for extracting the phenolic compounds.

The effect of ratio of ethanol to raw material on extraction yield of phenolic compounds is study. As ratio of ethanol to leaves powder increases, the extraction yield slowly increases first and a maximum yield achieved at 1:8 g/ml, and then slightly decreases after the ratio of ethanol to leaves powder exceeds 1:8 g/ml. This phenomenon may be attributed to the mass transfer principle and the distribution of ultrasonic energy density in the extraction solutions[15,25]. Lower ratio of ethanol to leaves powder has higher concentration gradient, leading to higher diffusion and extraction yield. But when the ratio is over 1:8 g/ml, the decrease of the distribution of ultrasonic energy density in the extraction solutions is dominant, and has a negative effect on the extraction yield[26]. Therefore, the ratio of ethanol to plant material of 1:8 g/ml is sufficient for extracting the phenolic compounds. The results show that antioxidant capacity ($DPPH^{\cdot}$, FRAP, O_2^- and NO values) of the extracts was the highest in 80 ml of solvent, which was in accordance with the phenolic contents of the extracts[27,28].

Time and temperature of extraction are important parameters to be optimized in order to minimize energy cost of the process. At every studied condition, the amount of extracted polyphenols increased continuously with the time[29]. The mechanism of the ultrasonic

extraction process here has two main stages. First, dissolution of soluble components on surfaces of the plant matrix occurs and secondly, mass transfer of the solute from the plant matrix into the solvent by diffusion and osmotic processes[30]. After 10 min, the slow extraction is observed by a low raise in both concentrations. Twenty minutes are accepted as the optimum time in this process, giving the equilibrium concentration of the extracted leaves. The total phenolic content of all samples increased steadily as a function of time. There was a tendency to drop of the antioxidant capacity after 20 min. This can be explained by the heating effect of overexposure to ultrasound treatment for longer extraction time, which leads to the degradation of antioxidant property of the extracts[23]. Therefore, the antioxidant capacity and anti-inflammatory property ($DPPH^{\cdot}$, FRAP, O_2^{-} and NO values) of the extracts was the highest in 20 of extraction time, which was in accordance with the phenolic contents of the extracts.

Generally, the temperature has a positive effect on the extraction of phenolic compounds from vegetal sources[31], The observed positive effect of temperature could be explained by the higher solubility of polyphenols in the solvent, the higher diffusivities of the extracted molecules and the improved mass transfer at higher temperatures. Although a higher temperature increased cavitation of ultrasonic assisted extraction by assisting in cell wall breaking in order to release the polyphenols, a higher temperature decompose phenolic compounds[32]. The effect of heating temperature to the polyphenols extraction from plant material was associated to the types and different bound forms of polyphenols that are presented in plants depending on the species . Hence, different plant species resulted in different optimum extraction and recovery of extracted compounds[33]. The ultrasonication temperature of 60 °C was the optimum temperature for the maximum yield of phenolic contents and antioxidant capacity of the leaves extracts. Previous studies have proved that application of heat increased mass transport phenomena by improving internal liquid phase pressure, resulting in centrifugal circulation of the solutes through plant membranes[34] . Furthermore, heat application is able to break the bonding of the phenolic-matrix and impact the membrane chemical structure of plant tissues and causing coagulation of lipoproteins[24].

In conclusion, The obtained results allowed optimizing the conditions for the extraction of antioxidant polyphenols from leaves of Ghars variety from *Phoenix dactylifera* L by using ultrasonic assisted extraction. Ultrasound assistance improves considerably both kinetics and yields of extraction of phenolics showing an efficient way to produce antioxidant-rich extracts at reduced time and high temperature. The effect of ultrasound is more significant while the operating conditions are generally less favourable for the extraction. The ethanol as solvent improved also greatly the extraction process. here is a very good correlation between the concentrations of polyphenols in the extracts and the corresponding antioxidant and anti-inflammatory activities. leaves of Ghars variety from *Phoenix dactylifera* L, often under-utilized part of this tree should thus be regarded as a potential source of natural antioxidant, and have potential to be developed as an ingredient in health or functional food. Further extensive scientific study into this rather abundant natural resource is warranted.

REFERENCES

1. Ignat I, Volf I, Popa VI. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chemistry 2011;126:1821–1835.
2. Gómez-Ordóñez E, Jiménez-Escrig A, Rupérez P. Effect of the red seaweed Mastocarpus stellatus intake on lipid metabolism and antioxidant status in healthy Wistar rats. Food Chemistry 2012;135: 806–811.
3. Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Câmara RBG, Nobre LTDB, Costa MSSP, Almeida-Lima J, Farias EHC, Leite EL, Rocha HAO. Biological activities of sulfated polysaccharides from tropical seaweeds. Biomedicine and Pharmacotherapy 2010; 64(1): 21–28.
4. Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology 2007; 17(5): 541–552.
5. Kang JY, Khan MNA, Park NH, Cho JY, Lee MC, Fujii H, Hong YK. Antipyretic, analgesic, and anti-inflammatory activities of the seaweed Sargassum fulvellum and Sargassum thunbergii in mice. Journal of Ethnopharmacology 2008; 116(1): 187–190.
6. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry 2006; 94: 550–557.
7. Yangthong M, Hutadilok-Towatana N, Phromkunthong W. Antioxidant Activities of Four Edible Seaweeds from the Southern Coast of Thailand. Plant Foods for Human Nutrition 2009;64:218–223.
8. Halliwell B, Aeschbach R, Löliger J, Aruoma OI. The characterization of antioxidants. Food and Chemical Toxicology 1995; 33:601–617.
9. Zhu K-X, Lian., Xiao-Na Guo C-X, Peng W, Zhou H-M. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. Food Chemistry 2011; 126: 1122–1126.
10. Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar RMNV. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. Journal of Controlled Release 2006;113: 189–207.
11. Perez-Jimenez J, Arranz S, Tabernero M, Diaz-Rubio ME, Serrano J, Goni I, Saura-Calixto F. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Research International 2008;41: 274–285.

12. Al-Farsi MA, Lee CY. Nutritional and functional properties of dates: A Review. Critical Reviews in Food Science and Nutrition 2008; 48: 877-887.
13. Baliga MS, Baliga BR, Kandathil SM, Bhat HP, Vayalil P K. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Research International 2011; 44: 1812-1822.
14. Laouini SE , Segni L, Ouahrani MR, Gherraf N, Mokni S. Scavenging effect, anti inflammatory and diabetes related enzyme inhibition properties of leaves extract from selected varieties of Phoenix dactylifera L. International Journal of Pharmacognosy and Phytochemical Research 2014; 6 (1): 66-73.
15. Liu S, Sun J, Yu L, Zhang C, Bi J, Zhu F, Qu M, Yang Q. Antioxidant activity and phenolic compounds of Holotrichia parallela Motschulsky extracts. Food Chemistry 2012; 134:1885-1891.
16. Omoruyi BE, Bradley G, Afolayan AJ. Antioxidant and phytochemical properties of *Carpobrotus edulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC Complement Alternative Medicine 2012;12:215.
17. Falleh H, Ksouri R, Oueslati S, Guyot S, Magné C, Abdelly C. Interspecific variability of antioxidant activities and phenolic composition in Mesembryanthemum genus. Food and Chemical Toxicology 2009; 47: 2308-2313.
18. Asadi S, Ahmadiani A, Ali Esmaeili A, Sonboli A, Ansari N, Khodagholi F. In vitro antioxidant activities and an investigation of neuroprotection by six Salvia species from Iran: A comparative study. Food and Chemical Toxicology 2010; 48: 1341–1349.
19. Kilani-Jaziri S, Bhouri W, Skandrani I, Limem I, Chekir-Ghedira L, Ghedira K. Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of Cyperus rotundus extracts. South African Journal of Botany 2011;77: 767-776.
20. Christudas S, Paul A, Chidambarm K, Savarimuthu I. In vitro antioxidant, antidiabetic and antilipidemic activities of Symplocos cochinchinensis (Lour.) S. Moore bark. Food and Chemical Toxicology 2012; 50: 1547-1553.
21. Makris DP, Boskou G, Andrikopoulos NK. Recovery of antioxidant phe-nolics from white vinification solid by-products employing water/ethanol mixtures. Bioresource Technology 2012; 98: 2963–2967.
22. Laouini SE , Segni L, Ouahrani MR, Gherraf N, Mokni S. Evaluation of Phenolic Content and Antioxidant Capacity of Ethanolic Extract for Selected Varieties of Phoenyx dactylifera. International Journal of Pharmacognosy and Phytochemical Research 2013; 5 (2): 79-83.
23. ŞahinS, Şamli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrasonics Sonochemistry 2013; 20:595–602.
24. Spigno G, De Faveri DM. Microwave-assisted extraction of tea phenols: aphenomenological study. Journal of Food Engineering 2009; 93:210–217.
25. Zeng ZL, Xi GX, Ge XH. Study on comparison of two extraction methods of polysaccharide from Momordica charantia L. vines. Science Technology and Food Industry 2013; 34: 285–308.
26. Zhao Z, Xu X, Ye Q, Dong L. Ultrasound extraction optimization of Acanthopanax senticosus polysaccharides and its antioxidant activity. International Journal of Biological Macromolecules 2013;59: 290–294.
27. Khan MK, Abert-Vian M, Fabiano-Tixier A, Dangles O, Chemat F. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel. Food Chemistry 2010; 119 :851–858.
28. The SS, Birch EJ. Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes. Ultrasonics Sonochemistry 2014; 21: 346–353.
29. Galvan d'Alessandro L, Kriaa K, Nikov I, Dimitrov K. Ultrasound assisted extraction of polyphenols from black chokeberry. Separation and Purification Technology 2012; 93: 42–47.
30. Veličković DT, Milenović DM, Ristić MS, Veljković VP, Ultrasonic extraction of waste solid residues from the Salvia sp. essential oil hydrodistillation. Biochemical Engineering Journal 2008; 13: 150–156.
31. Bucic-Kojic A, Planinic M, Tomas S, Bilic M, Velic D, J. Study of solid–liquid extraction kinetics of total polyphenols from grape seeds. Journal of Food Engineering 2007;81: 236–242.
32. Prasad KN, Yang B, Zhao MM, Ruenroengklin N, Jiang YM. Application of ultrasonication or high-pressure extraction of flavonoids from Litchi fruit pericarp. Journal Food Process Engineering 2009; 32(6): 828–843.
33. Settharaksa S, Jongjareonrak A, Hmadhlu P , Chansuwan W, Siripongvutikorn S, Flavonoid, phenolic contents and antioxidant properties of Thai hot curry paste extract and its ingredients as affected of pH, solvent types and high temperature, International Food Research Journal 2012; 19:1581–1587.
34. Corrales M, Toepfl S, Butz P, Knorr D, Tauscher B, Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. Innovative Food Science and Emerging Technologies 2008; 9:85–91