Rouiha Zohraet al. / Journal of Pharmacy Research 2016,10(1),1-7

Research Article
ISSN: 0974-6943

Available online through
www.jprsolutions.info

Evaluation of Phenolic Content and Antioxidant Capacity of leaf extract from *Phoenix Dactylifera L* obtained by different pH of aqueous extraction

Rouiha Zohra[a], Ouahrani Mohammed Redha[b], Laouini Salah Eddine[b]
[a] Laboratory of Valorization and promotion of Saharan resources , Kasdi Merbah University, Ouargla, Algeria
[b] laboratory of Valorization and Technology of Saharian resources, Hamma Lakhdar University, El-Oued, Algeria

Received on:10-10-2015; Revised on: 19-12-2015; Accepted on: 07-01-2016

ABSTRACT

Influence of pH aqueous medium extraction on phenolic content, flavonoid content, proanthocyanidins, phytochemical composition, antioxidant activities of leaves extract from *Phoenix dactylifera L* was investigated. It was observed that extraction yield increased at weak acidic condition (value of pH between 2 and 3), and the pH effects on the composition extraction process were illustrated. For the low value of pH, the yield extraction increase, similar phenomena for the antioxidant activity and concentration of individual phenolic compounds obtained by HPLC, but the phenolic content decrease. For the high value of pH, the yield extraction decrease, the low antioxidant activity and concentration of individual phenolic compounds obtained by HPLC, but the phenolic content increase. The optimum pH value of medium extraction solvent it is 2.

KEYWORDS: *Phoenix dactylifera* L, pH, antioxidant, DPPH, flavonoid, phenolic content

1. INTRODUCTION:

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. The fruits which are the most commonly used part are an important source of nutrition, especially in the arid regions where due to the extreme conditions, very few plants can grow. In conversational languages, dates are known as Sugar Palm (English), Nakhal (Arabic)[1]. Date palm (Phoenix dactylifera L.) is an important tree for the populations living in the Algerian Sahara. It is a vital component of their diet. This fruit has great importance from nutritional and economic points of view. Global production of date fruits exceeds 7 million metric tonnes annually in the world. During 2010, 710 000 metric tonnes were produced in Algeria which is the first producer in the Arab Maghreb union.

The chemical composition of date fruits was reported in many studies. These fruits are rich in simple sugars like glucose and fructose (65–80%), and represent a good source of fibers (6.4–11.5%) as well as some essential minerals (0.10–916 mg/ 100 g dry weight). In addition, dates are characterized by a low fat content (0.2–0.5%) and lack of starch [2]. Polyphenols are a group of naturally occurring secondary metabolites derived from shikimate-derived phenylpropanoid and polyketide pathways. They feature more than one phenolic ring and are classified based on the nature of their carbon skeleton: phenolic acids, flavonoids, stilbenes and lignans [3].

Humans consume polyphenols in their daily diet, as they are widespread in plant-based foods, estimated range of consumption being approximately 1 g per day. Cereals/whole grains contain numerous bioactive compounds mainly phenolic acids and flavonoids. Millet grain phenolics possess bioactivities against several pathophysiological conditions and may serve as potential natural sources of antioxidants in food and biological systems[4].

Polyphenols may have therapeutic health effects for a variety of chronic pathological conditions including cancer, neurodegenerative diseases, diabetes, and cardiovascular diseases. Many polyphenols are derived from natural food products. Thus, they are often considered safer and more easily integrated into lifestyle changes than conventional pharmaceutical drugs. Recently, specific molecular targets for various polyphenols have been discovered[5].

Due to their potential as health promoters and food preservatives, there is a need to maximize the extraction of phenolic compounds from plants material. Several extraction conditions, including tea particle size, brewing temperature, length of extraction, ratio of solvent, type

***Corresponding author.**
Dr. Laouini Salah Eddine,
Valorisation and Technology of Resource Saharian Laboratory,
El-Oued University, P.O. Box 789,
El-Oued, Algeria.

of solvent and the number of times the same material is extracted, have been found to directly affect the extraction efficiency of the extract constituents.

The low pH value of the extraction solution can prevent the oxidation of phenolics, while the use of low temperatures may preserve anthocyanin stability[6,7]. Thus, an investigation of the efficient extraction of phenolics from *Phoenix dactylifera L* requires evaluation under various temperatures and solvent pH values. Phenolic compounds show good antioxidant ability[8], but they are relatively unstable[9]. As the stability is dependent on various factors, such as pH value and temperature[10], the antioxidant ability of the phenolic compounds under the conditions of various temperatures and pH values also needs further evaluation.

The objective of this study was to examine the effects of various extraction pH values on the extraction yield of phenolics from *Phoenix dactylifera L* and then evaluate total antioxidant ability and scavenging activities (DPPH, FRAP and antioxidant capacity) and anti-inflammatory activity of leaf extract from *Phoenix dactylifera L* under various pH conditions.

This study we want demonstrate the potential antioxidant activity with Algerian leaves of *Phoenix dactylifera* L note that studies on this type of leaves is rare ,so that we can use these natural extracts as food additives in replacement of synthetic compounds.

2. MATERIALS AND METHODS

Plant material

The leaves of *Phoenix dactylifera* L were collected from south east of Algeria, state of El Oued on May 2013. The leaves were thoroughly washed and reduced into small pieces and were air dried in the laboratory at room temperature 30 °C for 8 days. The dried leaves powdered into particles (about 1 mm in size). 15 g of the powder was extracted with 150 ml of water (80-90 °C) for 5 h in Soxhlet in different pH (7 ;6 ;5 ;4 ;3 ;2) ; where the pH values were fixed by adding a solution of acetic acid to increase the acidity of pH either to lose, we added a solution of NaOH, At the end of the extraction, each extract was passed through Whatman No.1 filter paper (Whatman England). The filtrate obtained was concentrated in vaccum using evaporator and re-dissolved in the same extraction solvent (water) to prepare the required concentration for the subsequent essays[6].

Total phenolic content

The method described by using Folin-Ciocalteu reagent was used to determine total phenolics content in aqueous extract of S. latifolia bark. A volume of 0.5 mL of the extract (1 mg/mL), was mixed with 2.5 mL of 10% Folin-Ciocalteu and 2 mL of Na2CO3 (75% w/v). The resulting mixture was vortexed for 15 sec and incubated at 40 °C for 30 min for colour development. The absorbance of total phenolics was measured at 765 nm using Hewlett Packard, UV/visible light. Total phenolics content was expressed as mg/g tannic acid equivalent (TE) using the expression from the calibration curve Y=0.588 x + 0.004, R^2=0.996, where x is the absorbance and Y is the gallic acid equivalent in mg/g. The experiment was conducted in triplicate and the results were expressed as mean ±SD values[7].

Total flavonoids

The flavonoids content in extracts was determined spectrophotometrically using an aluminum chloride colorimetric method, based on the formation of a complex flavonoid aluminum. Each plant extract with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1 ml of 1 mol·L^{-1} potassium acetate (CH_3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm against the distilled water blank[8].

Total flavonoids content was expressed as mg/g rutin equivalents (RE) using the expression from the calibration curve Y=1.041 x - 0.369, R^2=0.995, where x is the absorbance and Y is the rutin equivalents in mg/g. The experiment was conducted in triplicate and the results were expressed as mean ±SD values.

Total proanthocyanidins

Total proanthocyanidins was determined based on the procedure of sun et al. The mixture of 3 ml of vanillin- methanol (4? v/v), 1.5 ml of hydrochloric acid was added to 0.5 ml (1 mg/ml) of aqueous extract and vortexed. The resulting mixture was allowed to stand for 15 min at room temperature and the absorbance was then measured at 500 nm. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the equation obtained from the calibration curve: Y=0.768X+0.139, R^2=0.996, where X was the absorbance and Y is the catechin equivalent (mg/g)[9].

DPPH assay

The radical scavenging activity of the extracts against DPPH radical was measured using the method of Brand-Williams et al[10], slightly modified as follow: an aliquot (1 mL) of methanolic solution containing different amounts of 0.01–1 mg/mL) were added to 2 mL of daily prepared methanol DPPH solution (0.1 mM). The mixture was shaken gently and left to stand at room temperature in the dark for 15 min. Thereafter, the absorbance was read at 515 nm. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution[11]. Results were expressed as radical scavenging activity percentage (%) of the DPPH radical according to the formula: %DPPH radical scavenging =$[(A_0-A_S)/A_0]*100$. Where A_0 is the absorbance of the control and A_S is the absorbance of the

sample. The effective concentration having 50% radical inhibition activity (IC50), expressed as mg extract/ mL, was determined from the graph of the free radical scavenging activity (%) against the extract concentration

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was performed based on the procedure described by Benzie and Strain (1996) with slight modifications[12]. In this assay, 100 µL of the diluted sample were added to 3 mL of the FRAP reagent and the reaction was monitored after4 min at 593 nm. The results were expressed as µmol Fe(II)/g fresh weight (FW) of vegetable.

Determination of antioxidant capacity by the phosphomolybdenum method

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method[13]. An aliquot of 0.1 ml of sample solution (equivalent to 100 lg) was combined with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In the case of the blank, 0.1 ml of methanol was used in place of sample. The tubes were capped and incubated in water bath at 95 _C for 90 min. After the samples were cooled to RT, the absorbance of the aqueous solution of each was measured at 695 nm[12]. The antioxidant capacity was expressed as an equivalent of ascorbic acid (mg ascorbic acid/g dried extract) using the expression from the calibration curve Y=4.171 x + 0.119, R^2=0.997, where x is the absorbance and Y is the antioxidant capacity in mg/g. The experiment was conducted in triplicate and the results were expressed as mean ±SD values.

HPLC analysis

The analysis of individual phenolic compounds was performed following the method proposed by Gonzlez-Gomez et al[6]. The chromatographic separation carried out with a Shimadzu HPLC C18 column (250 mm X 4.6 mm, 5 mL) and heated to 35 ° C. The mobile phase used for the separation consists of aqueous 0.1% TFA (A) and acetonitrile (B) defined in gradient mode as follows: initial conditions of 10% B; 0-3 min 10% B; 3-15 min 15% B; 15 to 20 minutes the composition was constantly maintained at 15% B; 20 to 25 min 18% B and 25 to 40 min 30% B. The period of the column equilibrium is 5 minutes. The flow rate was set at 1 ml / min for all experiments. The concentration of extract it is 10 mg / ml and the ability of the injection loop was 10 µl. Detection was performed at 300 nm. After each cycle, the system is reconditioned for 20 minutes before further analysis.

Statistical analyses: The data obtained in this study were expressed as the mean of three replicate determinations plus or minus the standard deviation (SD). Statistical comparisons were made with Student's test. *P* values <0.05 were considered to be significant.

RESULTS

Extraction yields and phenolic contents: Figure 1 shows the extraction yield (g/15 g dry weight). Generally, the pH= 2 afforded the highest yield (32.99±2.15w/w), while the lowest (15.85±4.22 and 16.19±5.34) was obtained from the PH=6 and PH=4 respectively. Intermediate values were found in the rest of pH.

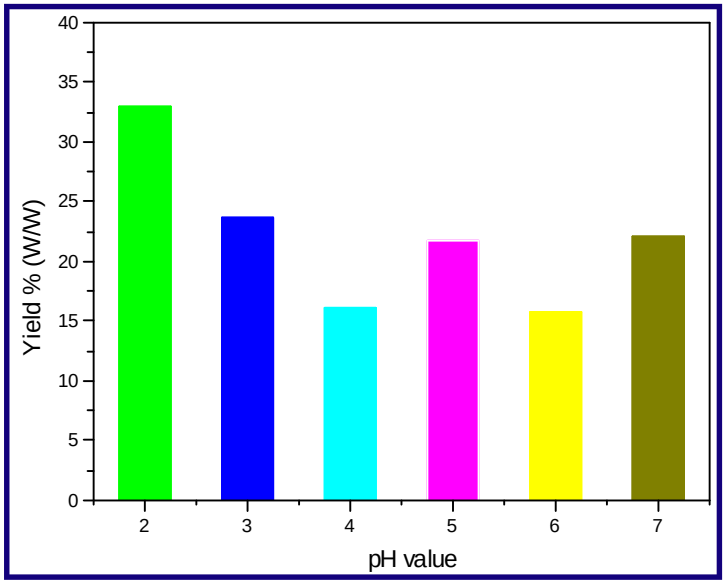


Figure1: Mass yield of leaves extract of *Phoenix dactylifera L* obtained by water in different pH

Figure 2 shows the TPC (mg GAE/ g of extract), TFC (mg rutin/ g of extract) and Total proanthocyanidins contents (mg catechin E/g of extract). Results of the analysis of polyphenols indicated that the pH=6 contained the highest concentration of polyphenols and flavonoids, While The pH =3 contained highest concentration of proanthocyanidins; whereas the pH = 2 contained the lowest.

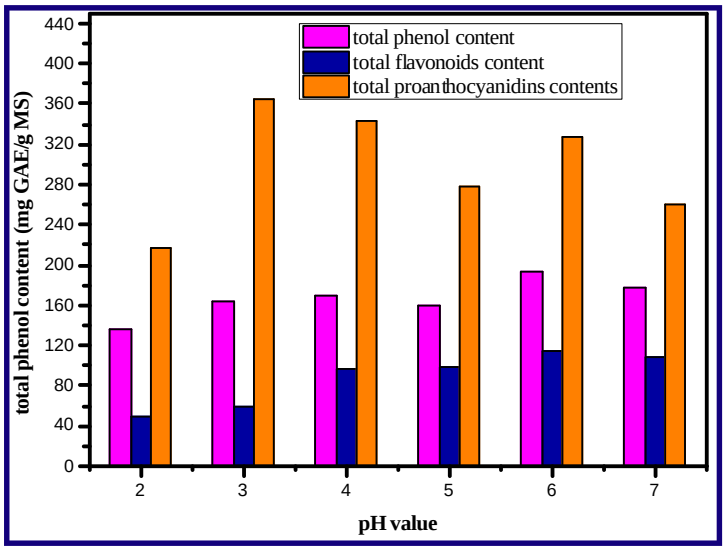


Figure 2: Total phenol content (TPC),total flavonoids content (TFC) and Total proanthocyanidins contents of leaves extract of Phoenix dactylifera L obtained by water in different pH

The DPPH radical is a stable radical with absorption band at 515–528 nm. It loses this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. Because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations, it has been extensively used for screening anti-radical activities of extracts. Results in figure 3 demonstrated that all samples exhibited dose-dependent DPPH radical scavenging activities. The pH= 6 (IC_{50} = 1.35±0.04mg/mL) has the most active scavenger of DPPH radical followed by leaves extract, then pH=2 (IC_{50} = 2.35±0.08 mg/mL). but the least scavenger of DPPH radical followed in pH=3 (IC_{50} = 3.17±0.03 mg/mL).

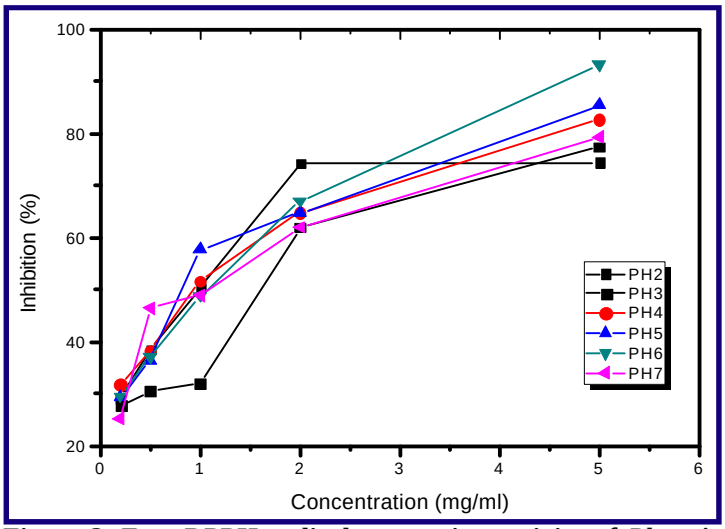


Figure 3. Free DPPH radical scavenging activity of *Phoenix dactylifera L* leaf extract obtained by different pH aqueous extraction

Ferric-reducing antioxidant power (FRAP) assay

The results for the selected extracts are presented in Table 1.the PH=5 has the best antioxidant capacity (168.04±1.86 µmol Fe II /g FW) while the pH =7 shows the least (129.27±1.77 µmol Fe II /g FW). Antioxidant capacity determined by phosphomolybdenum method: the results in table 1 shows that the highest antioxidant capacity observed in pH= 4 (68.34±0.71 mg ascorbic acid/g dried extract) whereas pH=2 has the smallest antioxidant capacity (55.11±0.60 mg ascorbic acid/g dried extract).

Table 1. Determination of antioxidant capacity by DPPH assay, Ferric-reducing antioxidant power (FRAP) assay and the phosphomolybdenum method

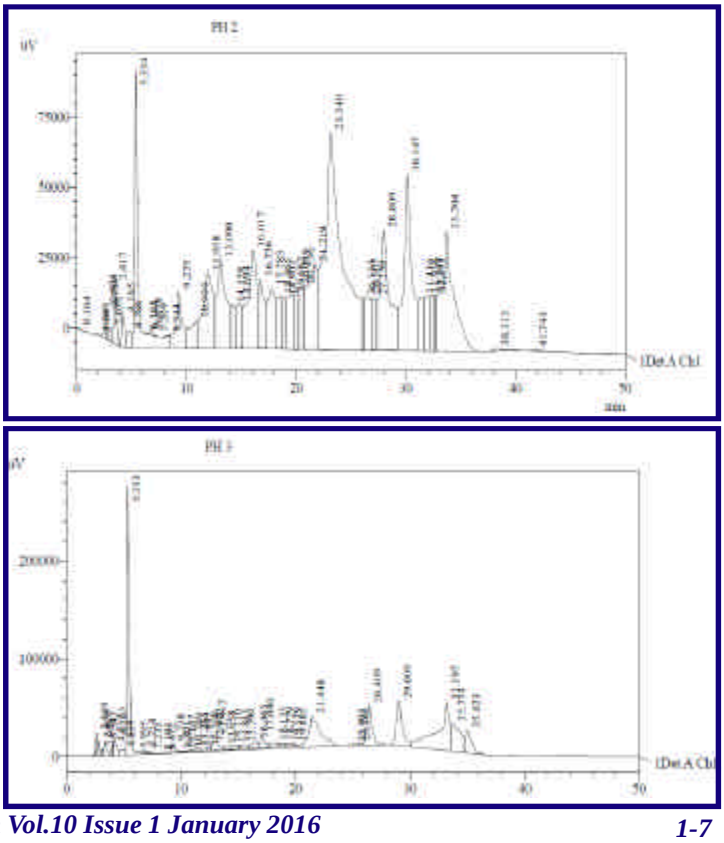
pH	DPPH IC_{50}= (mg/ml)	Ferric-reducing antioxidant power (µmol Fe II /g FW)	antioxidant capacity determined by phosphomolybdenum method (mg ascorbic acid/g dried extract)
2	1.35±0.04	147.02±1.82	55.11±0.60
3	3.17±0.03	156.96±0.30	55.92±0.60
4	2.50±0.02	164.73±4.48	68.34±0.71
5	1.99±0.05	168.04±1.86	63.62±0.69
6	2.35±0.48	158.31±5.15	64.12±1.08
7	2.71±0.02	129.27±1.77	62.81±0.73

HPLC assay: in table 2 the results present that pH= 7 has the lower concentration of phenolic composes and we observe that the rutin compose shows in pH 2,3,4,5 and 6 by high concentration which it arrive at 89.16 µg/mg Ms in pH = 4. Gallic acid and demonstrate highest concentration in pH 3 and 2 (20.91 and 10.01 µg/mg Ms) that is the same observation in vanillin which it results a concentration 2.72 and 2.31 µg/mg Ms in pH 3 and 2 at successively. The maximum concentration of ascorbic acid present in pH = 5 while chlorogenic acid and cafeic acid show the main concentration in pH = 2. These result give me an idea about pH =2 that it has the best medium extraction for the phenolic compounds.

Table 2: Constituents content of extract from *Phoenix dactylifera L* analyzed by HPLC

Components	Retention temps (mn)	Equation	Correlation Coefficient
			R^2=0.998
Gallic Acid	5.61	Y= 22781.36 X	R^2=0.998
Chloregenic Acid	13.49	Y=37492.06 X	R^2=0.999
Cafeic Acid	16.54	Y=70429.77 X	R^2=0.998
Vanilline	21.46	Y=80555.42 X	R^2=0.999
Rutine	28.49	Y=3118.94 X	R^2=0.998

Components	Concentration of phenolic compounds (µg/mg DW) PH					
	2	3	4	5	6	7
	0.99	1.36	1.85	2.10	1.27	0.21
Gallic Acid	10.01	20.91	1.83	0.71	2.40	0.30
Chloregenic Acid	4.86	0.08	1.93	-	0.73	0.02
Cafeic Acid	1.31	0.38	1.11	0.94	0.71	0.01
Vanilline	2.31	2.72	0.84	0.74	0.46	0.03
Rutine	82.68	54.02	89.16	9.69	58.65	0.005



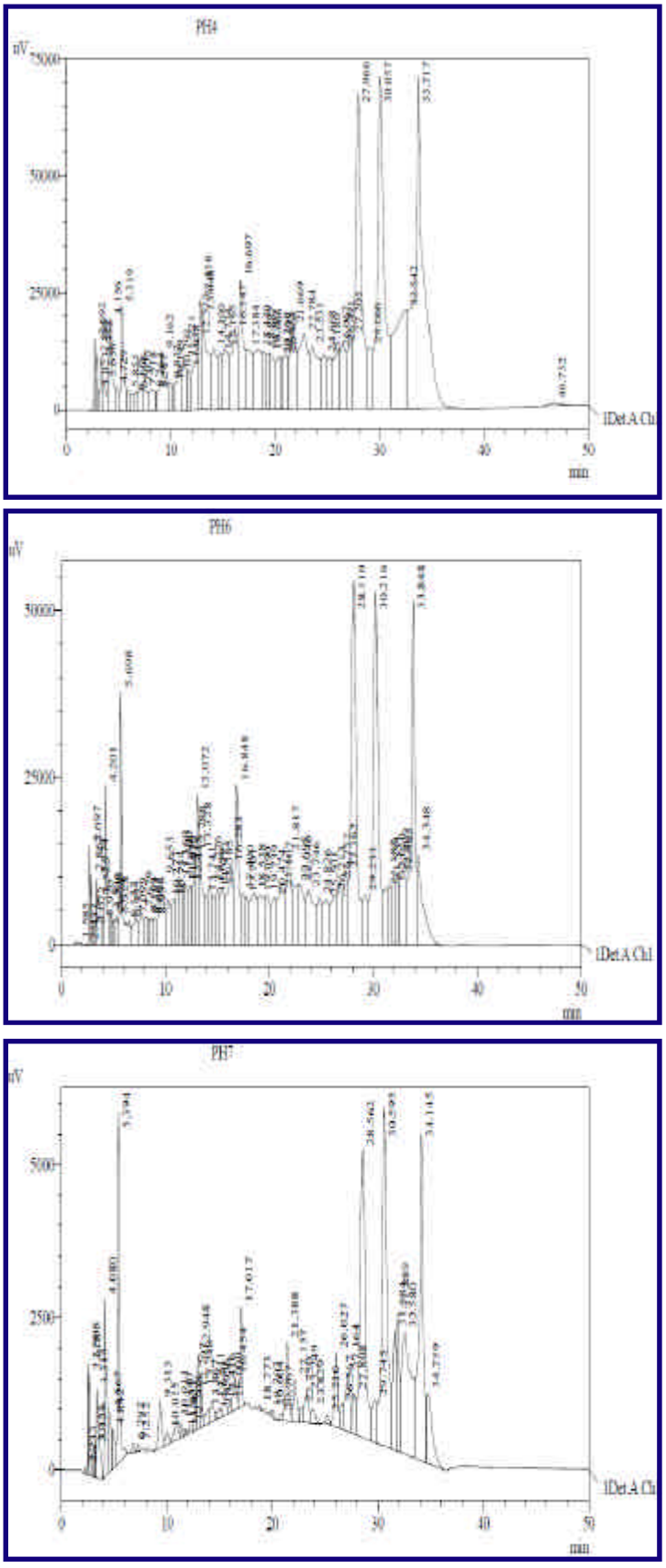


Figure 5. Chromatogram of phenolic components leaf extract from *Phoenix dactylifera L* obtained by different pH aqueous extraction

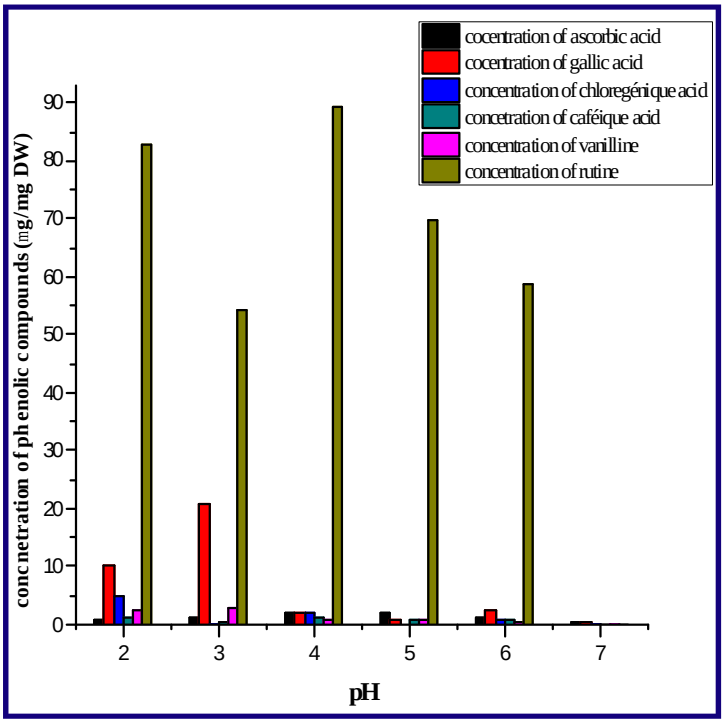


Figure 6: Quantification of phenolic components by HPLC

DISCUSSION

Most plants including lettuce show maximum phenolic compounds activity at or near neutral pH values[14,15]. pH is a determining factor in the expression of enzyme-atic activity. It alters the ionization states of amino acid side chains or the ionization of the substrate. One reason for the loss of antioxidant activity at pH 7 could be higher activity of phenolic compounds at this pH resulting in higher phenol degradation. Phenolicoxidase catalyzes the oxidation of phenolic such as chlorogenic to form dark condensation products[16]. pH of the surrounding medium influence the radical scavenging capacity of these phenolic compounds[17]. pH dependent increase in the antioxidant activity against lipid oxidation and stable radicals can, at least in part, be related to the deprotonation of an OH moiety. The actual mechanism of antioxidant action of the deprotonated forms can be either hydrogen atom or electron donation or both.

The flavonoid, total phenolic contents and antioxidant activity of extracts as effect of pH are estimated. The result indicated that at slightly acidic pH 2, flavonoid, total phenolic content and DPPH radical scavenging activity were highest. It meant that these active compounds derived from the paste were easily destroyed by high acidic. However, it confirmed that flavonoid and total phenolic content in the curry paste play an important role for DPPH radical scavenging activity as mentioned above. The antioxidant activities of different extracts indicate strong dependence of DPPH radical scavenging activity on the pH of the system. The antioxidant activity of different extracts from *Phoenix dactylifera L* was higher at pH=2, this results

is similar to the results founded by Librán et al[18], the best value of pH extraction for the antioxidant activity at pH = 2. These differences may be due to different samples used and various compounds being extracted in each case.

Experimental results show that the lower pH will be helpful for obtaining higher extraction yield as shown in figure1and it provide highest yield as we found in figure 1 and these result showed in other researche[13]. Table 1 demonstrate the influence of pH at concentration of different phenolic compounds that we found pH =3 has the highest concentration of phenolic components. The same result shown for the inhibition scavenging of DPPH radical of leaves extract. whereas, it is found from pH =2 does not show higher total phenol content, total flavonoids content and total proanthocyanidins contents of leaves extract. Previous works indicated the degree of correlation between antioxidant activity and polyphenol contents depends not only on the total polyphenol content, but also on the composition of extracts[19].

By the above investigation, we can say that lower pH will decrease the phenolic compounds but decrease the extraction performance.

CONCLUSION

This study reflects the importance of controlling the studied extraction value of pH to obtain an extract with the highest polyphenol content and with an adequate antioxidant activity.

Using water as extracted solvent was a good promising for phenolic content and antioxidant activity. However, if pH of testing system was higher than 4, it reduced more flavonoid, total phenolic contents and but increase antioxidant activity. Even, lower pH degraded the curry paste quality in term of flavonoid, total phenolic contents and proanthocyanidins. In general, the best process conditions for pH of medium extraction solvent it is 2. Further studies are in progress in this laboratory for the isolation and identification of the bioactive compounds.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Tohami Lanez, Director of Valorization and Technology of resource Saharian laboratory (El-Oued University, Algeria) for supported and his laboratory assistance.

REFERENCES

1. Manjeshwar Shrinath Baliga , Bantwal Raghavendra Vittaldas Baliga , Shaun Mathew Kandathil ,Harshith P. Bhat , Praveen Kumar Vayalil. A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.). Food Research International 2010; 44: 1812–1822
2. zahia benmeddour, emira mehinagic, dominique le meurlay, hayette louaileche. Phenolic composition and antioxidant capacities of ten Algerian date (phoenix dactylifera l.) cultivars: a comparative study. journal of functional foods 2012; 5:346 –354
3. Huong T.T. Phan , Tsuyoshi Yoda , Bindu Chahal , Masamune Morita , Masahiro Takagi , Mun'delanji C. Vestergaard. Structure-dependent interactions of polyphenols with a biomimetic membrane system. Biochimica et Biophysica Acta 2014;1838: 2670–2677
4. Gavirangappa Hithamani, Krishnapura Srinivasan. Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum). Food Chemistry 2014; 164: 55–62.
5. Hae-Suk Kim a, MichaelJ.Quon c, Jeong-aKim. New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. RedoxBiology 2014;2:187–195.
6. Jonathan Delgado Adámez, Esther Gamero Samino, Esperanza Valdés Sánchez, David González-Gómez. In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (Vitis vinifera L.). Food Control 2011;24, 136-141
7. Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. Asian Pacific Journal of Tropical Biomedicine 2012; 118-124
8. Ivana Karabegovi, Milena Nikolova, Dragan Veli kovi, S ša Stoji evi, Vlada Veljkovi and Miodrag Lazi. Comparison of Antioxidant and Antimicrobial Activities of Methanolic Extracts of the *Artemisia* sp. Recovered by Different Extraction Techniques. *Chinese Journal of Chemical Engineering*, 2011; 19(3): 504-511
9. Sunday Oyewole Oyedemi, Anthony Jide Afolayan. Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of Schotia latifolia Jacq. Asian Pacific Journal of Tropical Medicine ,2011; 952-958
10. Selma Dziria, Imed Hassenb, Saloua Fatnassia, Yassine Mrabeta, Herve Casabiancac, Belgacem Hanchid, Karim Hosnia. Phenolic constituents, antioxidant and antimicrobial activities of rosy garlic (Allium roseum var. odoratissimum). Journal of functional foods
11. Gui-Fang Denga, Xi Lina, Xiang-Rong Xub, Li-Li Gaoa, Jie-Feng Xiea, Hua-Bin Lia. Antioxidant capacities and total phenolic contents of 56 vegetables. Journal of functional foods 2012

12. Amin Ardestani, Razieh Yazdanparast. Inhibitory effects of ethyl acetate extract of Teucrium polium on in vitro protein glycoxidation. Food and Chemical Toxicology 2007;45:2402 2411
13. Panliang Zhang, Hui Zhang, Kewen Tang , Jianmin Yi, Yan Huang.(2015). Influence of pH on enantioselective extraction of aromatic acid enantiomers in centrifugal contactor separators: Experiments and simulation. Separation and Purification Technology 2015;141: 68–75
14. Arzu A, Vural Go, Leif HS H dependent antioxidant activity of lettuce (L. sativa) and synergism with added phenolic antioxidants. Food Chemistry 2016; 190: 25–32.
15. Dogãn S, Salman U. Partial characterization of lettuce (Lactuca sativa L.). Polyphenol oxidase. European Food Research and Technology 2006; 226(1-2): 93–103
16. Howard LR, Brownmiller C, Prior RL. Improved color and anthocyanin retention in strawberry puree by oxygen exclusion. Journal of Berry Research 2014; 4: 107–116.
17. Borkowski T, Szymusiak H, Glýszcynska-Swiglo A, Rietjens I MCM, Tyrakowska B. Radical scavenging capacity of wine anthocyanins is strongly pH-dependent. Journal of Agricultural and Food Chemistry 2005; 2005(53): 5526–5534.
18. Celia M L, Luis M, Esperanza M. GC, Daniel VB. Polyphenol extraction from grape wastes: Solvent and pH effect. Agricultural Sciences 2013; l4 (9) B: 56-62.
19. Spigno G, De Faveri DM. Antioxidants from grape stalks and marc: Influence of extraction pro- cedure on yield, purity and antioxidant power of the ex- tracts. Journal of Food Engineering 2007; 78: 793-801.

Source of support: Nil, Conflict of interest: None Declared