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Research Article

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Effects of extraction solvents on bound phenolic contents and antioxidant activities of Tantboucht dates(*Phoenix dactylifera* L.) from Algeria

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ABSTRACT

The extraction of phenolic compounds from edible partof date palm (Phoenix dactylifera L.)fruit (DPF) from Algeria, namely Tantboucht (Tnb) was optimised using four different solvent. Total phenolic content (TPC) and total flavonoid content (TFC) of the DPF were measured using Folin–Ciocalteau and aluminum chloride colorimetric methods, respectively. The antioxidant activity of different extracts was carried using DPPH radical scavenging activity and reducing power. These results showed that date had strongly scavenging activity on DPPH .The IC50 value for DPPH radical scavenging activity was 0.09mg/ml in acetone/H_2Oextract.And also, acetone/H_2O extract showed the best reducing power.

Key words: Solvent; Date; Phenolic content; Flavonoid content; Antioxidant activity; DPPH.

INTRODUCTION

In the recent years, increasing attention has been paid to the role of diet in human health. Epidemiological studies have shown that high fruit and vegetable consumption has health benefits in the prevention of chronic diseases, such as atherosclerosis and cancer, cardiovascular, cataract, diabetes, coronary heart diseases, and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases[1]. Antioxidants may reduce the risks of these diseases and improve general human health[2]. Date palm (*Phoenix dactylifera* L.) is an important fruit for the populations living in the Algerian Sahara. It is a vital component of their diet[3].The chemical composition of date fruits was reported in many studies[1, 4, 5]and that date fruit extract has strong antioxidant and antimutagenic properties[6].

The Objective of this study was to compare antioxidant activity and phenolic contents of the date palm with four different solvents.

EXPERIMENTAL SECTION

2.1Date sample

One ripe date palm (*Phoenix dactylifera* L.) fruit variety (DPF)Tantboucht (Tnb) was harvested in October 2013. Fruit was collected from the Touggourt region (Algeria), the fruit was sectioned and their seeds were carefully removed and stored in paper bags.

2.2Preparation of the extracts

After washing with water and removing the seeds, the edible part of date cut to small pieces using a scissors and dried at room temperature. The extraction of antioxidant compounds from the date cultivar was carried using four different solvents as described byAl-Farsi, Alasalvar et al[5], with slight modifications. Five grams of sample were mixed for 24 h with 50 ml of H_2O, absolute methanol, methanol/H_2O (8/2), or acetone / H_2O (7/3) at room temperature and with agitation.

The mixture was centrifuged at 3500 × g for 15 min, and the supernatant was filtered using filter paper and then evaporated to dryness using a rotary evaporator. The extracted phenolics were dissolved in 10 ml of methanol. Methanolic solutions of phenolic were kept frozen until analysis.

2.3Total phenolic content(TPC)

Total phenolic content (TPC) of the date extracts was determined using theFolin–Ciocalteu method [5]. The different concentration (200µl) of extracts were mixed with 1.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) for 5 min at room temperature. 1.5 ml of aqueous sodium bicarbonate (60 g/l) was added, and the mixture was vortexed and allowed to stand at room temperature. After 90 min, the absorbance was measured at 725 nm. The TPC was determined from standard gallic acid curve and expressed as milligrams of gallic acid equivalent per 100 g of dry weight of date for three replicates (mg GAE/100 g DW).

2.4Total flavonoid content (TFC)

Total flavonoid content (TFC) of the date extracts was performed according to the aluminum chloride colorimetric method [7].Five hundred microliters of the different concentration of extracts were added to 150 µl sodium nitrite solution (5%) followed by 300µl aluminum chloride (10%). Test tubes were incubated at room temperature for 5 min, and then 1 ml of 1 M sodium hydroxide was added. The absorbance of the mixture was determined at 510 nm. The TFC was determined from standard quercetin curve and expressed milligrams of quercetin equivalents per 100 g of dry weight of date for three replicates (mg QE/100 g DW).

2.5Antioxidant activity assays

2.5.1 Ferric reducing power (FRP)

The reducing power of the date extracts was assayed according to the method of Kumaran and Joel Karunakaran[8], with slight modifications. Each extract (1 ml) was mixed with 2.5 ml of phosphate buffer (0.2 mol/l, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50 ◦C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added to the mixture. 2.5 ml of solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$), and the absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicates increased reducing power of the sample. All analyses were carried out in triplicate. Reducing power was expressed as millimolar (mM) ascorbic acid equivalents antioxidant capacity (AEAC).

2.5.2 DPPH radical scavenging capacity

The scavenging activity of the extracts was determined using DPPH-scavenging assay according to the method explained by Govardhan Singh, Negi et al[9], with slight modifications. 150 μl of Different concentrations of the extract were added to 3.0 mL 0.1 mM DPPH solution in methanol. After mixing vigorously the tubes were incubated in dark. After 30 min the absorbance was read at 517 nm. IC50 value (the concentration required to scavenge 50% DPPH free radicals).

The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect $(\%) = ((A_0 - A_1)/A_0) * 100.$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample.

2.6 Statistical analysis

All analyses were carried-out in triplicate and the results were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

3.1 Total phenolic content (TPC)

Extraction with solvents is frequently used for the isolation of antioxidant compounds, and both extraction yield and antioxidant activity of the extracts have a strong relationship with the solvent employed, mainly due to the different polarity of the compounds obtained[10]and the solubility of this compound in the solvent used for the extraction process[11, 12]. Therefore, it is hard to select an appropriate solvent for the extraction of phenolic contents from all samples. In this study, different solvents such as acetone/H_2O (7/3), methanol/H_2O (8/2), methanol and H_2O have been used for the extraction of phenolic compounds.

The effects of these solvents in extracting polyphenols were shown in table 1. These results showed that the TPC varied greatly among different solvents. This indicated the possible influence of extracting solvent on total phenolic contents.

Among all the extracts, methanol was found to be the most efficient solvent for extracting phenolic compounds when compared with all other solvent systems used, the level of these compounds ranged from84.51 to163.93 mg GAE/100 gDW.

Figure 1 shows the TPC results of DPF extract from four types of solvents. Methanol showed the highest extraction capacity for phenolic from DPF in comparison to the other solvents in this order: methanol >acetone/H_2O (7/3) >methanol/H_2O (8/2) > H_2O.

3.2 Total flavonoid content (TFC)

Among all the extracts, methanol was found to be the most efficient solvent for extracting flavonoid compounds when compared with all other solvent systems used, the level of these compounds ranged from1.85 to6.83 *mg* QE/100 g DW(Table 2).

Figure.2 shows the TFC results of DPF extracts from four types of solvents. Methanol showed the highest extraction capacity for flavonoids from DPF in comparison to the other solvents in this order: methanol > acetone/H_2O (7/3) > methanol/H_2O (8/2) > H_2O.

Table 1- Total phenolic content (TPC) Effect of solvent on phenolic content (mg GAE/100 DW) in Tnb

Phenolic content (mg GAE/100 g)				
Solvent Sample	Acetone/H_2O (7/3)	Methanol/H_2O (8/2)	Methanol	H_2O
Tnb	159.92±16.25	158.84±1.95	163.93±4.11	84.51±2.98

Table 2- Total flavonoid content (TFC)

Effect of solvent on flavonoid content (mg QE /100 DW) in Tnb

Flavonoid content (mg QE /100 g)				
Solvent Sample	Acetone/H_2O (7 /3)	Methanol/H_2O (8/2)	Methanol	H_2O
Tnb	6.51±0.13	3.59±0.02	6.83±0.09	1.85±0.04

Figure. 1. Total phenolic content (mg GAE / 100 g DW)

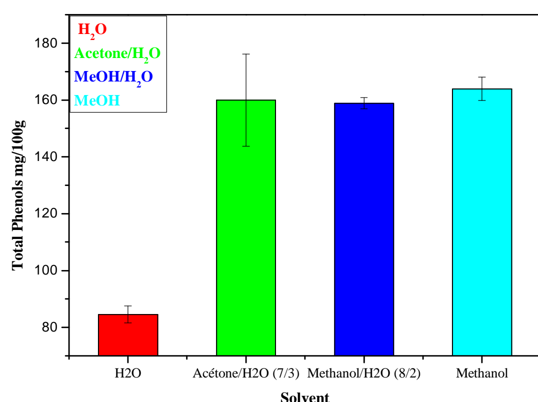
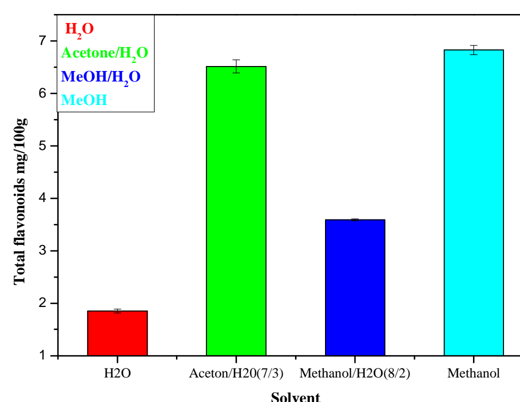


Figure. 2.Total flavonoid content (mg QE / 100 g DW)



3.3 Antioxidant activity

3.3.1 Ferric reducing power (FRP)

The reducing power of a compound can be assessed by the reduction of $Fe^{3+}$of the ferric cyanide complex [$FeCl_3$/$K_3Fe(CN)_6$]to the ferrous (Fe^{2+}) form by donating an electron. Therefore, Fe^{2+}can be monitored by measuring the formation of Perl's Prussian blue at 700 nm[13] **.**

The AEAC of the date extract using the different solvents was presented in figure. 3. Results showed that the AEAC of Tnb was the highest 7.52 mM for acetone/H_2O (7/3). However, H_2O had been lower AEAC than the other solvent3.07mM(Table 3).

3.3.2 DPPH radical scavenging capacity

DPPH, a stable radical, is used to evaluate samples' ability of providing proton. Absorbance at 517 nm decreased as DPPH radical was scavenged with a phenomenon that the solution color turned purple into light yellow[14].DPPH radical was scavenged by antioxidants through donation of hydrogen to form a stable DPPH molecule[14].

In order to identify the most suitable solvent for assessing antioxidant capacity, the antiradical activity(DPPH test) was evaluated either using the pure solvents or varied mixtures.

In the current study, the ability of test samples to scavenge DPPH radical was assessed on the basis of their IC50 values.

As shown in figure. 4, the acetone/H_2O (7/3) and H_2O extracts from Tnb, exhibited the highest scavenging activity on DPPH radicals than methanol/H_2O (8/2) extract. However, the methanol solvent exhibited the less scavenging activity on DPPH radicals.

From this study, we found that acetone/H_2O (7/3) extract from Tnbhad better scavenging ability on DPPH radicals as the value of IC50 is 0.09 mg/ml (Table 3), whereas the amount of TPC was weak (159.92 mg GAE/100 g DW).

These results showed that radical-scavenging activity differs not only by the concentration of phenolic compounds but also with degree of hydroxylation and polymerization[15-17].

Table 3 - Antioxidant capacities of one Algerian date cultivars. Tantboucht (Tnb)

IC50 DPPH (mg/ml)				
Solvent Sample	Acetone/H_2O (7 /3)	Methanol/H_2O (8/2)	Methanol	H_2O
Tnb	0,09±0.03	0,21±0.00	0.22±0.01	0.10±0.00
Reduction power AEAC (mM)				
Solvent Sample	Acetone/H_2O (7 /3)	Methanol/H_2O (8/2)	Methanol	H_2O
Tnb	7.52±0,10	5.64±0.29	6.54±0.01	3.07±0.10

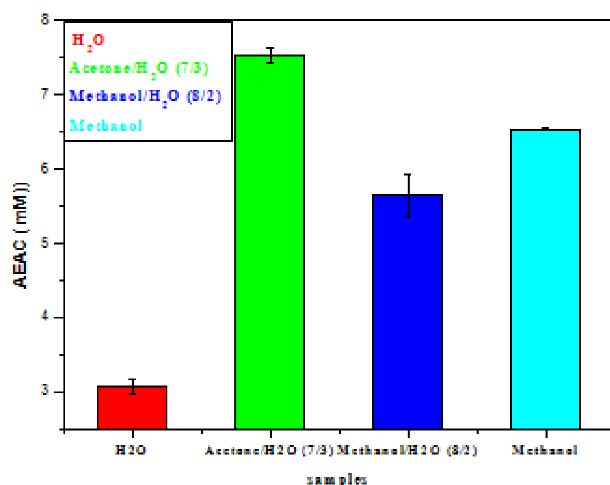


Figure. 3. AEAC values of ascorbic acid equivalents antioxidant capacity of extracts

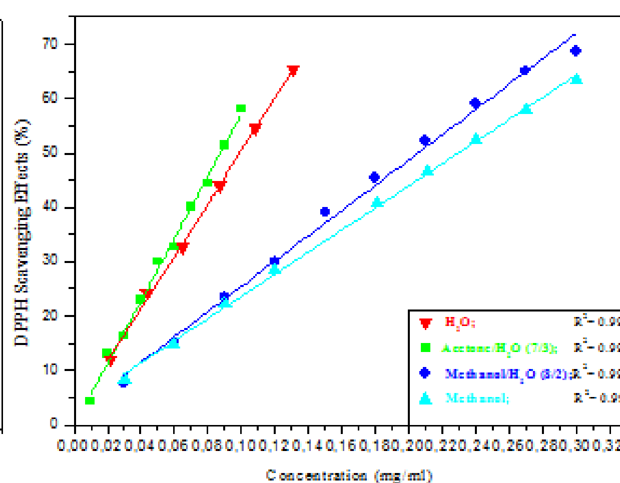


Figure. 4.Concentration–response plots for inhibition of the absorbance of DPPH radical at 517 nm for DPF extracts

CONCLUSION

The influence of the solvent (H_2O, absolute methanol, methanol/H_2O (8/2) and acetone / H_2O (7/3)) on the phenolic compounds, flavonoids and the antioxidant properties of the DPF extracts was demonstrated. Methanol was the most efficient solvent for extracting phenolic compounds and flavonoids from the DPF, while acetone/H_2O (7/3) presented the highest antioxidant activity DPPH free radical and reducing power when compared with all other solvents. These results showed that phenolic compounds are the main micro constituents contributing to the antioxidant activity of the DPF.

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