COMPARISON OF ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF THREE VARIETIES OF ALGERIAN DATES

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Abstract- The date palm is a typical plant of the arid and semi arid areas. Unfortunately, not all of its varieties profits from an economic interest except Deglet-Nour. This poses a danger to date palm genetic inheritance and the biodiversity. This study was initiated to investigate the antioxidant of methanol-water extracts from three date palm fruits (DPF) common in the region of Ouargla (Algeria); Degla Baidha (DB), Tamjhourt (Tam) and Tafezauine(Taf). The antioxidant capacities of these varieties were evaluated by using different methods, namely $ABTS^{\cdot+}$(2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)) scavenging activity expressed as Trolox equivalent antioxidant capacity (TEAC), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, Reducing power expressed as Ascorbic acid equivalents antioxidant capacity (AEAC). Total phenol content (TPC) was determined by using Folin–Ciocalteau Reagent. Total flavonoid content (TFC) was determined by using aluminum chloride method. Concerning the ABTS scavenging activity (Trolox equivalent), the TEAC values decreased in the order of DB>Tam>Tef. Effective scavenging concentration (IC50) on DPPH radical decreased in the order of Tam>DB>Tef. Effectiveness in reducing power was in a descending order of Tam>DB>Tef. The TPC was found from 41.8 to 84.73 mg/100g gallic acid equivalents. The order of TPC of DPF is: DB>Tef>Tam, while TFC varied between 7.52 to 14.1 mg/100g rutin equivalents and in a descending order of DB>Tam >Tef. These results suggest that all date varieties serve as a good source of natural antioxidants and could potentially be considered as a functional food or functional food ingredient.

Keywords: date palm fruit; antioxidant capacity; ABTS; DPPH; reducing power.

ETUDE COMPARATIVE DE L'ACTIVITE ANTIOXYDANTE ET LE CONTENU EN COMPOSÉS PHÉNOLIQUES DE TROIS VARIÉTÉS DE DATTES COMMUNES D'ALGERIE

Résumé- Le palmier dattier est une plante typique des zones arides et semis arides. Malheureusement, ce ne sont pas toutes les variétés de dattes qui bénéficient d'un intérêt économique, sauf pour la variété Déglet-Nour. Elle représente donc un danger pour le patrimoine génétique et la biodiversité. La présente étude recherche à évaluer l'activité antioxidant des extraits aqueux méthanoliques de trois variétés de dattes communes: Dégla Baidha (DB), Tamjhourt (Tam), et Tafezauine (Tef), de la région de Ouargla (Algérie). Les capacités antioxydantes de ces variétés ont été évaluées en utilisant différents méthodes, dont l'$ABTS^{\cdot+}$ (l'acide 2,2 '-azino-bis-(3-éthylbenzthiazoline-6-sulfonique), balayage activité, exprimée en Trolox Equivalent Antioxydant a Capacité (TEAC), DPPH (2,2-diphényl-1-picrylhydrazyl)) comme radical libre, le pouvoir réducteur exprimée en acide ascorbique équivalent antioxydant a capacité (AEAC). Les phénols totaux ont été déterminés avec le réactif de Folin-Ciocalteu. Pour les flavonoïdes totaux (TFC), il est utilisé la méthode du chlorure d'aluminium. L'activité antiradicalaire ABTS (Trolox Equivalent) et les valeurs de TEAC présentent une diminution selon l'ordre suivant: DB> Tam> Tef. L'effet scavenger de la concentration (I50) sur le radical DPPH diminue en ordre suivant: Tam>DB>Tef. L'efficacité du pouvoir réducteur du fer décroissant est selon l'ordre suivant: Tam>DB>Tef. Le TPC varie de 41,8 à 84,73 mg/100g équivalents acide gallique, l'ordre du PTC de DPF est: DB>Tef>Tam. Cependant le TFC varie de 7,52 à 14,1 mg/100g équivalents rutine selon l'ordre décroissant suivant: DB>Tam>Tef. Les résultats obtenus dans la présente étude laissent apparaître que toutes les variétés de dattes constituent une bonne source d'antioxydants naturels et pourraient être considérés comme un aliment fonctionnel ou ingrédient d'aliment fonctionnel.

Mots clés: fruits du palmier dattier; capacité antioxydant; ABTS; DPPH; pouvoir réducteur.

Introduction

In 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 [1,2], as well as inflammation and problems caused by cell and cutaneous aging [3].

The date palm (*Phoenix Dactylifera*) is a monocotyledon of the family of the Palmae, one of the genera of which are the Coryphoideae, of which one species is Phoenix_Dactylifera [4], cultivated mainly in North Africa but also in South Asia, USA and Australia. It covers a surface of about 800.000 ha and it is important directly or indirectly for the life of about 100 millions of inhabitants [5]. It offers a good food source of high nutritional value. This tree gives many date growing countries in remote areas, the main food for a considerable number of people and provides working conditions to considerable numbers of laborers in rural areas [6].

Dates are important crops in the southern regions of Algeria, where the estimated annual production is 468000 tons from an area of 140000 hectares planted with date palms [7]. More than 940 cultivars have been currently identified [8]. In the Wilaya of Ouargla,for instance, date palm trees cover an area palm of 20 622 hectares with a total number of palms of 2 341034 producing about 849082 tones in 2007 [9].

The aim of this study is to investigate *in vitro* the antioxidant capacities of the methanol-water extracts from three date palm fruit (DPF) varieties from the Ouargla region (Algeria). In the present study, methanol-water extracts were prepared from dried and powdered of plant material. The antioxidant activity was examined for all the three varieties using tow antioxidant assays such as, free radical scavenging, reducing power. Furthermore, the total phenolic content and flavonoids contents were also measured from plant extracts.

1.- Materials and method

1.1.- Plant material

Three different Algerian ripe date palm fruit (DPF) varieties, Degla Baidha variety(DB) harvested on November 9th 2006, Tamjhourt variety(Tam) was harvested on October 11th 2006, Tafezauine variety(Taf) was harvested on October 29th 2006, fruits were collected from the Ouargla region (Algeria), the fruit were segmented and their seeds were carefully removed, and stored in paper bags in a refrigerator.

1.2.- Chemicals and reagents

Chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland), Sigma-Aldrich (Steinheim, Germany and Merck (Germany).Riedel-dhaen,Prolabo.

1.3.- Sample preparation and extraction

The plant material was air dried until dryness at room temperature in the dark for three weeks, and milled to a fine powder using a coffee-grinder. The phenolics from samples were isolated by a modified version of the method described by Djerridane et al (2006) [10]. 20 g of fine ripe date palm fruit powder macerated in 100 ml methanol:water (80:20, v/v) for 24 h at

room temperature. The crude preparation was filtered, and the residue re-extracted twice with 50 ml of the same hydrau-alcoholic solvent for 24 h at room temperature. The extract was filtered. The filtrates were combined. After removing the alcohol under vacuum at 40 °C, the Phenolic compounds were extracted three times with ethylacetate (1:1, v/v). The three organic phases were combined; the residual water in the ethylacetate was eliminated with anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The extracted phenolics were dissolved in methanol and then filtered using filter paper. Methanolic solutions of phenolic were stored in a freezer for analysis. The storage conditions (time and temperature) were the same for all types of fruit.

1.4.- Determination of antioxidant capacities

1.4.1.- $ABTS^{\cdot+}$ scavenging activity

The $ABTS^{\cdot+}$ was prepared by enzymatic oxidation of ABTS with H_2O_2 and peroxidase [11], a green bluish complex was formed by mixing peroxidase, hydrogen peroxide, ABTS and solution PBS buffer (pH 6.8), the absorbance at 416 nm was recorded each minute after initial mixing. Appropriate solvent blanks were run in each assay, and all measurements are done at least 5 min. The results are expressed as the Trolox equivalent antioxidant capacity (µM TEAC).

1.4.2.- DPPH radical scavenging activity

Scavenging Radical activity of DPF extracts against stable $DPPH^{\bullet}$ (2-diphenyl-2-picrylhydrazyl hydrate) was determined using the method of Brand-Williams, Cuvelier, & Berset,(1995) [12]. This method was determined spectrophotometrically. When $DPPH^{\bullet}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light-yellow) were measured at 517nm on a UV-visible light spectrophotometer (UV-1601). The antioxidant activity of the extract was expressed as an IC50 value defined as the concentration (in mg/l) of the extract that inhibited the formation of DPPH radicals by 50% [10].

1.4.3.- Reducing power

The reducing power of DPF extracts was determined according to the method of Oyaizu (1986) [13]. Different concentrations of extracts were mixed with phosphate buffer and potassium ferricyanide . The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid was added to the mixture. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.
Reducing power (P_R) was expressed as Ascorbic acid equivalents antioxidant capacity (mM AEAC).

1.5.- Determination of total phenol content (TPC)

The concentration of total phenolics (TPC) was determined by the Folin–Ciocalteau colorimetric method [14]. The total phenolic content (TPC) was expressed as gallic acid equivalents (GAE) in mg/100 g dry plant material.The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard gallic acid graph.

1.5.- Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined according to the aluminum chloride

colorimetric method described of Chang, Yang, Wen, and Chern (2002) based on the method of Woisky and Salatino (1998) [15,16]. With slight modifications and results were expressed as mg rutin equivalents (RE) per 100 g of dry weight. The method is based on the quantification of yellow color produced by the interaction of flavonoids with $AlCl_3$ reagent. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard rutin graph.

2.- Results and discussion

2.1.- Determination of antioxidant capacities

2.1.1.- $ABTS^{\cdot+}$ scavenging activity

The chromogen 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) radical ($ABTS^{\cdot+}$) can be directly generated by the enzymatic system formed by hydrogen peroxide and horseradish peroxidase. The reaction is time- and concentration-dependent, and the $ABTS^{\cdot+}$generated shows excellent stability. The method is an adaptation of the decolouration method previously reported [17].

Fig. 1 illustrates the effects of the durational reaction of DPF on the suppression of the absorbance of the $ABTS^{\cdot+}$. To understand the effects of DPF concentration on the ABTS assay, the radical scavenging activities of the three DPFs were measured (fig. 2). The results showed that there was a concentration-dependency in the $ABTS^{\cdot+}$ radical scavenging activity for all DPFs. The plots suggest that at lower concentrations, the relationship between concentration and the decrease in the Absorbance is linear too. The antioxidant activity measurements of DPF. The TEAC values decreased in the order of DB>Tam>Tef.

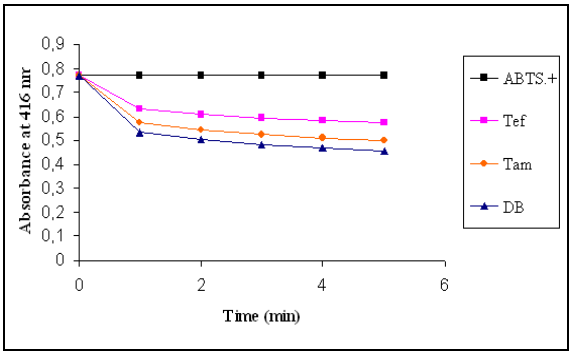

Fig. 1.- Plot of absorbance fall with respect to the concentration of plant extras (1–5 min)

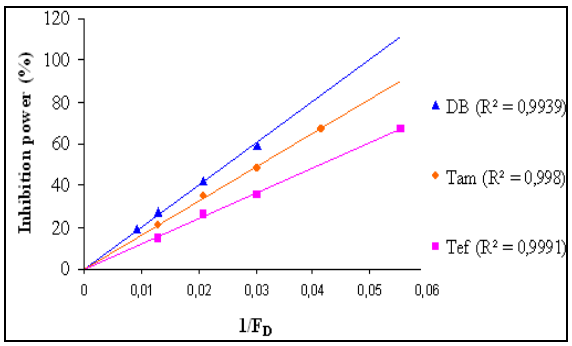

Fig. 2.- Concentration-response curves for inhibition of the absorbance of $ABTS^{\cdot+}$cation at (416 nm) for DPF extracts

2.1.2.- DPPH radical scavenging activity

DPPH$^{\bullet}$ radical is a stable lipophilic free radical which has been generally used for estimating antioxidant activity of food and medicine materials. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a colour change from purple to yellow [18]. In the current study, DPPH percent scavenging activities of plant crude extracts were measured in different concentrations ran and results are given for DPPH percent radical scavenging activity (% RSA) versus extract concentrations in g/l in figure 3. The scavenging effect was increased with increasing concentration.

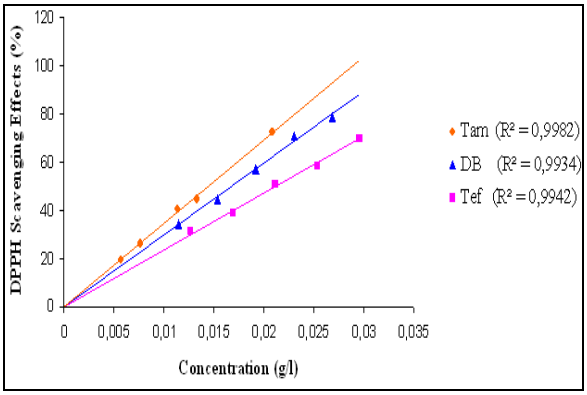

Fig. 3.- DPPH scavenging effect of DPF extracts

2.1.3.- Reducing power activity

Tanaka *et al.*, Duh have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts [19,20]. The antioxidant potential of the DPF was further investigated through its reducing power. Figure 4 show the reducing power of different DPF using the potassium ferricyanide reduction method Compared to ascorbic acid standard curve. For the measurements of the reductive ability, it has been investigated from the Fe^{3+}–Fe^{2+} transformation in the presence of extract samples. Highest activity was found in Tam, followed by DB and the lowest activity was found in Tef. The reducing power might be due to hydrogendonating ability, and is generally associated with the presence of reductones [20].

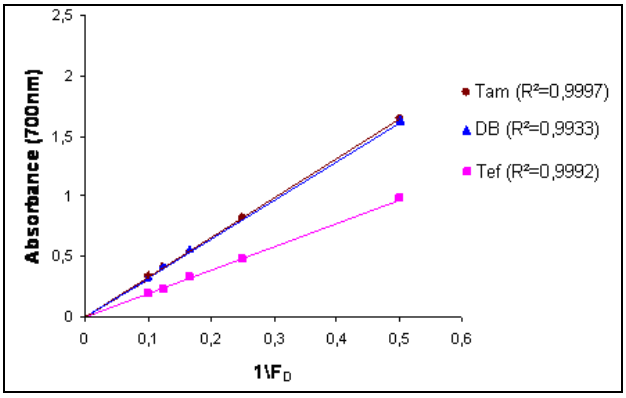

Fig. 4.-Reducing power effect of DPF extracts

2.3.- Total polyphenol and flavonoid contents

2.3.1.- Total phenolic content

The DB had the highest phenolic content, with 84.73 mg gallic acid equivalents/100 g dw sample, while lowest content was measured for Tam with 41.8 mg. The order of TPC of DPF is: DB >Tef >Tam.

2.3.2.- Total flavonoid content

Most of the flavonoids possess strong antioxidant properties following chain breaking mechanism. The highest TFC was observed for DB followed by Tam and Tef, respectively. A controversial order relative to other methods of antioxidant activity evaluation was observed.

The antioxidant activities of fruits (dates) of the date palm can be contributed to phenolic compounds, such as cinnamic acids and flavonoids (flavones, flavonols and flavanones) [20,21]. Thus, the DPPH radical scavenging activity of FDP extracts may be mostly related to their phenolic hydroxyl group. The concentration of hydrogen peroxide in water may vary according to the phenolic compounds. Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of H_2O_2–H_2O [23].

Conclusion

In this study, it was demonstrated for the first time that the methanol-water extracts from three date palm fruit varieties from the Ouargla region (Algeria) possessed a good antioxidant activity, which may be associated with their alleged health benefits.

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