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Phytochemicals Levels and Antioxidant Capacities of Figs Flowers Fruits

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Abstract

Since antiquity, phenolic compounds produced by plants were known as free radical scavengers and as powerful antioxidants. Huge interest has been made by researchers to the traditional uses of medicinal plants against illnesses related to oxidative stress. This study measures the correlation that can be existed between the antioxidant capacity and phytochemicals levels of four varieties of *Ficus carica* fruits, figs flowers or "*Bakor*" as called locally in Algeria. Therefore, extracts were assessed for determining their antioxidative potentials using both test of total antioxidant capacity and DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging test followed by quantitative phytochemical analysis to estimate the total flavonoid level (TFL), the total phenolic level (TPL), the total anthocyanin level (TAL) and the condensed tannins level of plants methanolic extracts. A positive correlation was observed between phenolics content and the antioxidant capacity of figs flowers methanol extracts. The methanolic extract of Bechar (*MeOH Var.2*) chelated 87. 9± 1.23 % of the DPPH free radical with IC50 value equal to 0.185 mg/g DW. A high antioxidant ability of almost all extracts is, probably, related to the appreciable rates of flavonoids, phenolics and tannins showed by those fig extracts. The highest value of phenolics level was detected among the variety 1 methanolic extract of Bechar (*MeOH Var.1*) of 10.4 mg GAE/g DW.

Keywords: Antioxidant capacity; *Bakor*; figs flowers; *Ficus carica*; Methanol extracts; DPPH.

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Introduction

Phytochemicals, including phenolics, flavonoids, flavonols, ascorbic acid, lignin, xanthones, stilbenes, etc., are plant-based secondary metabolites, which are associated with the protection of human health against chronic diseases [1, 2, 3, 4]. The relative importance of medicinal and food plant species can be assessed by their use-value. Plant species with more traditional uses exhibit high use value compared to those which have fewer ones [5]. Nowadays, medicinal plants considered as an important source of drugs as about 25% of the drugs prescribed worldwide derive from plants [6].

Fig tree *Ficus carica* Linn. originated in the Middle East areas such as Syria, Asia Minor, and Iran, then, it was spread to the Mediterranean basin countries by old humans [7, 8]. It belongs to the family of *Moraceae*. *F. carica* L. is one of the unique widely spread *Ficus* species that has edible fruits with high commercial value. The production of commercial fig is situated in regions that possess a Mediterranean climate [9].

F. carica L. has three figs yields, Early fig stays on the tree; Late fig of autumn or figs flowers carries from August to winter and is locally known as Bakor and the green or winter figs [10, 11]. Oxidative stress is an inequality between prooxidants and antioxidants in favor of the first contributing to the appearance of several pathologies. The uncontrolled oxygen species resulted will have serious and severe consequences for the human organism [12]. Several studies focus on natural antioxidant sources to find new effective, safe and cheap antioxidants as there is a strong relationship between the decrease of certain chronic diseases and plants-produced antioxidants [13]. Fruits are essential functional foods that maintain the human vital functions as they providing a well-balanced diet [14]. Viewing the biological properties of *F. carica* fruits, our study focuses on the correlation between phytochemicals contents and antioxidant capacity of dried fruits methanolic extracts of figs flowers or "Bakor" originated of four different varieties of *F. carica*. Two varieties of Bechar and the two others from Mascara.

Materials and Methods

Collection of Plant Samples

Fruits from four different varieties of *F. carica* figs flowers or "*Bakor*" as called locally were collected between May and August 2018. Two varieties from Bechar located at the Southwest of Algeria: Var.1 (Lahmar), Var.2 (Ouakda). The other two ones from Mascara situated at the North of Algeria: *Var.3* (El Bordj), *Var.4* (Ghriss). Plants specimens were identified by the Laboratory of Biotoxicology, Pharmacognosy and Biological Valorisation of Plants (University of Saida).

Figure 1. Fruit of *Ficus carica* L. (Left: whole fruit; Right: cross-section) [15, 16].

Preparation of Methanolic Extracts

Four samples of fig flowers were air-dried and crushed. After, 1 g of *F. carica* fruits was soaked, under ultrasound, using pure methanol (20 mL) for 24 h. Plants extracts were filtered, concentrated and stored at 4°C until used (*MeOH Var.1, MeOH Var.2, MeOH Var.3, MeOH Var.4*) [17].

Phytochemical screening

Chemical products and reagents purchased from Merck Company, Darmstadt, Germany and phytochemical screening tests were repeated for three times.

Determination of percentage yield

The percentage yield was calculated for each extract using the formula:

Percentage yield (%) = a/b × 100

Where: (a)=the dry weight of extract, (b)=soaked samples material [18].

Determination of Total Flavonoids Level (TPL)

To measure the total flavonoids level, volumes of 2 mL from both plant extracts and 2% ethanolic solution of aluminum trichloride ($AlCl_3$) were mixed and incubated 10 min at room temperature. After measurement of absorbance at 430 nm, TFL resulted in mg quercetin per g dry weight [19].

Determination of Total Phenolic Level (TPL)

Total phenolics level was estimated via Folin–Ciocalteu's reagent. A mixture of sodium carbonate solution (2 mL, 2%) and 0.1 mL of plant extract was incubated for 5 min. Then, a volume of 100 µL Folin–Ciocalteu's reagent was added. The mixture was incubated for 30 min and absorbance was read at 700 nm to determine the TPL as mg gallic acid per g dry weight (mg GAE/g) [20].

Determination of Total Anthocyanins Level (TAL)

The pH differential method was used to deduce total anthocyanins level, by which, the absorbance of the reaction solution was measured at both 510 nm and 700 nm at two pH 1.0 then pH 4.5 using buffer systems: hydrochloric acid (0.2 M) and sodium acetate (1 M).

$A= (A_{510}-A_{700})_{pH1,0} - (A_{510}-A_{700})_{pH4,5}$

TAL=[(A x MW x DF) / MA] x 100

A: absorbance; MW: molar mass; DF: dilution factor; MA: molar absorption.

TAL was expressed as mg cyanidin-3-glucoside per g of dry weight (mg C3G/g) [2, 21].

Determination of Condensed Tannins Level (CTL)

The vanillin test was used to estimate the Condensed Tannins Level (CTL). The mixture contained methanolic solution of vanillin (4%, w/v), methanol (37%, v/v), HCl (8%, v/v), at equal volume was kept at 30 ° C until used. Then, a solution of 1500 µl of vanillin methanolic solution was added to 50 µl of plant extracts and 750 µl of concentrated HCl was incubated for 20 min. The absorbance was read at 550 nm and compared to a blank of equal volumes of both 37% methanol and 8% HCl. Results were expressed in mg of catechin equivalent per gram of the dry weight (mg CE/g) [22, 23, 24].

Tests of antioxidant capacity

The antioxidant capacity of the four varieties were evaluated using free radical scavenging test (DPPH) and total antioxidant capacity (TAC).

DPPH free radical scavenging test

DPPH scavenging test was used to estimate the capacity of each extract to scavenge hydrogen atom generated of 2,2-diphenyl-1-picrylhydrazil radical. A mixture of 1 mL of 100 µM methanol solution of the free radical DPPH and different concentrations of each extract was incubated 20 min. The absorbance of the solution was read at 517 nm and compared to the blank that contained both 1 mL of DPPH methanolic solution and 0,1 mL of methanol solvent [25, 26]. The inhibition percentage (% IP) of DPPH solution was estimated by the following formula:

$\% IP = [(At_0 - At_{20}) / At_0 \times 100)]$

Where, At_0= Absorbance of the blank solution after 20 min. At_{20}= Absorbance of each sample after 20 min. Then, the concentration of an extract that allowing to 50% inhibition of DPPH solution (IC50) was obtained graphically. The less is the IC50, the higher is the antioxidant capacity. A positive control with various concentrations was prepared by the methanolic solution of ascorbic acid [27].

Total Antioxidant Capacity (TAC)

The test of phosphomolybdenum reagent allows the determination of Total Antioxidant Capacity (TAC) of plant samples. A mixture that contains a 3 mL volume of a solution of ammonium molybdate reagent (4 mM), sulphuric acid (0.6 M) and sodium phosphate (28 mM) added to a volume 0.3 mL of each extract was incubated at 95°C. After, 90 min, the absorbance was measured at 695 nm against a control solution prepared under the same conditions, constituted of 0.3 mL of methanol and 3 mL of all the reagents used before. Total Antioxidant Capacity expressed as mg ascorbic acid per g dry weight (mg AAE / g). A calibration curve with various concentrations was prepared using ascorbic acid [28].

Statistical analysis

The experimental data obtained from the TFL, TPL, TAL, CTL and antioxidant capacity tests were expressed as means ± standard deviation. Statistical analysis of data was performed using Microsoft Excel. Statistical differences were estimated, One-way ANOVA and student's t-test were used. The correlation coefficient of antioxidant capacities as determined by the Pearson test. Values are to be statistically significant at $p < 0.05$.

Results and Discussion

Total flavonoids level, total anthocyanins level, total phenolics level, condensed tannins level of fig flowers fruits (*Bakor*) methanolic extracts are presented in **Figure 2**.

The total phenolics level of the four extracts varied from 4.7 to 10.4 mg GAE/g DW. The highest level was significantly ($p<0.05$) conferring to the variety 1 methanolic extract of Bechar (*MeOH Var.1*) and the lowest in the extract *MeOH Var.3* of Mascara (See **Figure 2**).

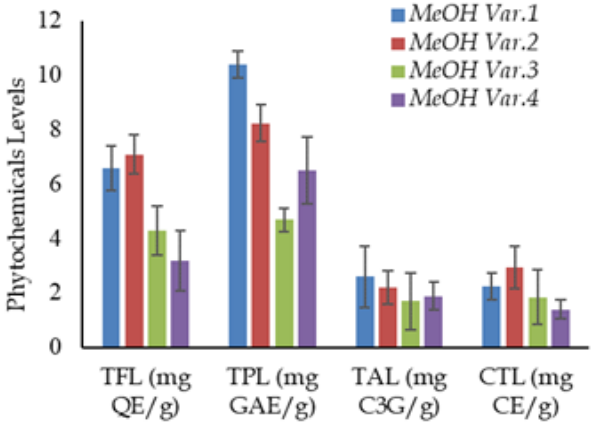

Figure 2. Levels of various phytochemicals of fig flowers fruits methanolic extracts. *MeOH Var.1; MeOH Var.2*: *Variety 1, 2* Bakor (Fig flowers) of Bechar. *MeOH Var.2; MeOH Var.3*: *Variety 2* Bakor of Mascara. TFL: Total Flavonoids Level, TAL: Total Anthocyanins Level, TPL: Total phenolics Level. CTL: Condensed Tannins Level. mg QE/g : quercetin equivalents; mg C3G/g : cyanidin-3-glucoside equivalents; mg GAE /g : acid gallic equivalents; mg CE/g: catechin equivalent.

The extract of Bechar *MeOH Var.2* has the highest Total flavonoids value 7.1 mg QE/g DW while *MeOH Var.4* has the lowest one of 3.2 mg QE/g DW. An important total anthocyanin levels were detected in *MeOH Var.1* of 2.6 mg C3G/g DW compared to the methanolic extract of mascara *MeOH Var.3* which has a low value (1.7 mg C3G/g DW). A statistical significant differences existed on phytochemicals levels between plant samples ($p < 0.05$).

Total phenolics amounts resulted in *F. carica* skin extracts ranged between 28.6 and 211.9 mg GAE / 100 g FW and between 24 and 237 mg GAE / 100 g FW [21, 29]. The considerable phytochemicals levels obtained can be explained by the sonication extraction using pure methanol as solvent and as such, is not an ignored proposition as the extraction of flavones, polyphenols, anthocyanins, tannins can

be very good using alcohol such as methanol solvent [30].

Furthermore, the special climate conditions such as the **low** monthly **rainfall** and the **high temperature** characterizing the harvest year of plant samples can also affect widely their phytochemicals amounts. According to Vallejo et al. 2012, the skin of the early fig fruit (first crop) is richer in phenolic components than late fruit (second crop) possibly as a consequence of climatic factors [31].

Figures 3, 4 and **5** illustrate the total antioxidant capacity and the % inhibition of DPPH.

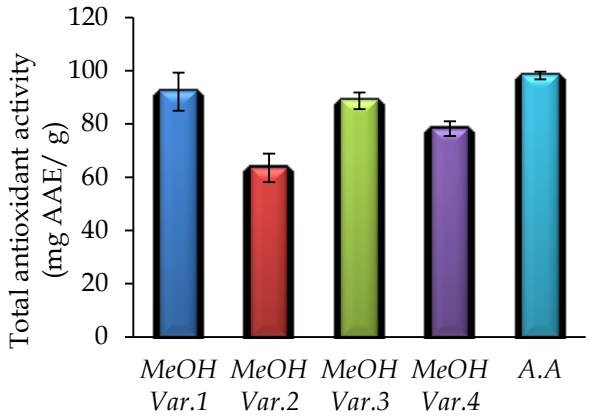

Figure 3. Total antioxidant capacity of fig flowers fruits methanolic extracts. *MeOH Var.1; MeOH Var.2*: *Variety 1, 2* Bakor (Fig flowers) of Bechar. *MeOH Var.3; MeOH Var.4*: *Variety 3, 4* Bakor of Mascara.

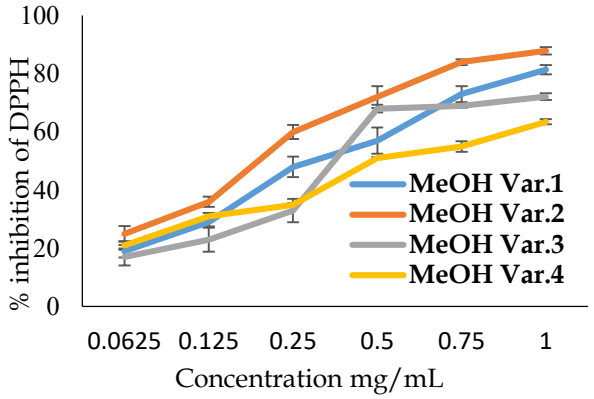

Figure 4. DPPH radical scavenging activity of fig flowers fruits methanolic extracts. *MeOH Var.1; MeOH Var.2*: *Variety 1, 2* Bakor (Fig flowers) of Bechar. *MeOH Var.3; MeOH Var.4*: *Variety 3, 4* Bakor of Mascara

The inhibition concentration value that exhibits 50% of radical scavenging activity for different varieties of extracts (**Table 1**). **Table 2** summarizes the correlation coefficients among antioxidant tests, total flavonoid level, total anthocyanins level and total phenolic level.

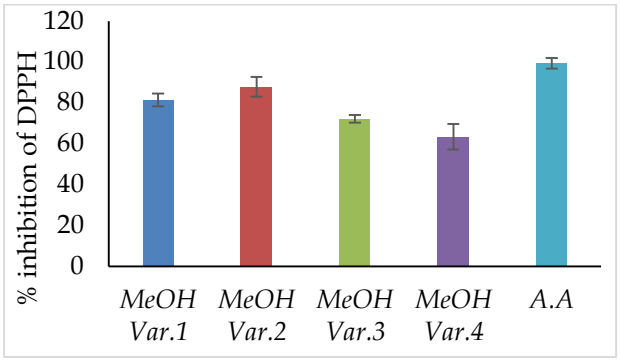

Figure 5. % inhibition of DPPH. MeOH Var.1; MeOH Var.2: Variety 1, 2 Bakor (Fig flowers) of Bechar. MeOH Var.3; MeOH Var.4: Variety 3, 4 Bakor of Mascara

Compared to the positive control IC50 value 0.004 mg/g DW, the lowest IC50 equal to 0.185 mg/g DW was deduced for methanolic extract of Bechar (*MeOH Var.2*), which chelated 87.9± 1.23 % of the DPPH free radical. However, the extract *MeOH Var.4* has presented the lowest %IP of 63.5 ± 0.87% with an IC50 value of 0.448 mg/g DW. Our results showed a statistically significant difference between studied extracts and positive controls ($p < 0.05$).

Table 1. IC50 value of fig flowers fruits methanolic extracts.

Varieties of fig flowers (Bakor)	IC50 (mg/mL)
MeOH Var.1	0.260^{A}
MeOH Var.2	0.185^{B}
MeOH Var.3	0.345^{C}
MeOH Var.4	0.448^{D}
A.A	0.004^{E}

MeOH Var.1; MeOH Var.2: *Variety 1, 2* Bakor (Fig flowers) of Bechar. *MeOH Var.3; MeOH Var.4*: *Variety 3, 4* Bakor of Mascara. *A.A: ascorbic acid.* Capital letters (A–B) and lowercase letters (a–b) indicate significant differences at $p < 0.05$.

The high TPL and TFL levels measured can explain widely such a considerable percent of inhibition. Phenolics are the most effective antioxidants, which function as free radical scavengers, and absorb oxygen radicals [32, 33], because of their acidity, ability to transfer electrons and characteristic benzene rings [34]. Solomon et al. 2006 have proven that, compared to the fruit pulp, fruit skins have the most contribution to phytochemicals amounts and antioxidant capacity and both dark- and brown-colored fig varieties are the most effective ones by their important amounts of flavonoids, polyphenols, and anthocyanins.

Besides, anthocyanins compounds of the dark- and brown-colored fig skin varieties contribute to 36 and 28% of their total antioxidant capacity [35].

Table 2. The correlation coefficient among antioxidant capacity tests, total flavonoid level, total anthocyanins level, total phenolic level, and tannins level.

MeOH Var.1	DPPH	TAC	*MeOH Var.3*	DPPH	TAC
TFL	0.812^{A}	0.531^{a}	**TFL**	0.954^{A}	0.208^{a}
TPL	0.873^{A}	0.715^{b}	**TPL**	0.943^{B}	0.681^{b}
TAL	0.601^{A}	0.224^{c}	**TAL**	0.176^{C}	0.566^{c}
CTL	0.730^{A}	0.478^{d}	**CTL**	0.658^{D}	0.619^{b}
MeOH Var.2	**DPPH**	**TAC**	***MeOH Var.4***	**DPPH**	**TAC**
TFL	0.914^{A}	0.574^{a}	**TFL**	0.507^{A}	0.288^{a}
TPL	0.948^{B}	0.724^{b}	**TPL**	0.426^{A}	0.397^{b}
TAL	0.632^{C}	0.587^{c}	**TAL**	0.311^{A}	0.141^{c}
CTL	0.972^{D}	0.598^{d}	**CTL**	0.529^{A}	0.336^{b}

TFL: Total Flavonoids Level, TAL: Total Anthocyanins Level, TPL: Total phenolics Level, CTL: Condensed Tannins Level, TAC: Total antioxidant capacity. *MeOH Var.1; MeOH Var.2*: *Variety 1, 2* Bakor (Fig flowers) of Bechar. *MeOH Var.3; MeOH Var.4*: *Variety 3, 4* Bakor of Mascara. Capital letters (A–B) and lowercase letters (a–b) indicate significant differences at $p < 0.05$.

The total antioxidant capacity ranged from 63.5 to 92.1 mg AAE/g dw for *MeOH Var.2* and *MeOH Var.1*, respectively in comparison with ascorbic acid 98.2 ± 1.43 mg AAE/g dw.

The total antioxidant capacity estimated for methanolic extracts of fig varieties fruits from the same region (Mascara) as our studied fig-flowers varieties 3, 4 (El Bordj, Ghriss) was ranged from 68.8 to 88.6 mg AAC/g DW and the highest values were observed for the ethanolic extracts. Also, the max of the total tannin content of methanolic extracts was max of 122.35 mg AAE/g DW [36].

According to the statistical analysis, separately, there are non-significant differences between the two fig varieties of Bechar: *Var.1* (Lahmar), *Var.2* (Ouakda) and also no significant differences between fig varieties originated of mascara *Var.3* (El Bordj), *Var.4* (Ghriss) ($P > 0,05$).

A positive and strong correlation was registered between the antioxidant capacity of plant extract and its total phenolic composition [37, 38, 39, 40, 41]. In vitro tests have shown that dried fig fruits possess a significant antioxidant capacity subsequently to their human consumption. As mentioned previously, the total antioxidant capacity correlated well with anthocyanins and phenolic compositions (R = 0.989, R = 0.515), but the correlation is low with the flavonoid amount R = 0.248 [15, 42]. However, the existence of other unidentified molecules with antioxidant properties in those extracts can not be overlooked. Peel extracts of fig fruits had a higher ability to scavenger free radicals, at all concentrations than pulp extracts with IC50 values of 80.04 and 28.85 mg/mL. For the pulp, IC50 values were 105.85 and 176.88mg/mL [43]. Results showed a positive and strong correlation between antioxidant capacity and different phytochemical compounds amounts. Phenolics and tannins components of MeOH Var.2 have an appreciable correlation with DPPH radical test R2 =0.948 and R2 =0.972, respectively. Total phenolics had a strong correlation with the DPPH radical test (R2 =0.948). Furthermore, an important relation between both total flavonoids and total phenolics and DPPH radical presented by the extract MeOH Var.3 with R2 =0.954 and R2 =0.943, respectively. A high correlation coefficient was deduced between antioxidant capacity and the levels of polyphenols and anthocyanins with R2 = 0.985 and R2 =0.992, respectively [34]. The differences observed in the antioxidant capacity of plant extracts can be related to certain parameters like those that the solvent used for extraction, its polarity, and the tests used [44]. Moreover, as the mechanisms of antioxidant effect are dissimilar and the most natural antioxidants are multifunctional, it is important to use various antioxidant capacity tests to take a global observation of it.

Conclusion

The important antioxidant capacity of methanolic extracts of fig flowers fruits is impressive and probably it is the result of their exceptional richness in phenolic compounds. Such bioactive molecules reacting as natural antioxidants and then, they are well known to display a positive impact on human health and can be considered for future uses as antioxidant components in agro-food industries. The Algerian flora known for its high richness and biodiversity as well as Algerian folk medicine is also considered as an appreciable source of both new drugs and bioactive molecules since ancient times. Future studies will be needed to elucidate more and more medicinal plants and traditional preparations used for therapeutic purposes.

Conflict of Interest

The authors declare that they have no competing interests.

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