Mycopath (2018) 16(2): 91-99 **Research Article**

Correlation between phenolic amount and antioxidant activity of bioproducts of *Ficus* varieties

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Abstract

Herbs renaissance is happening all over the world and they are staging a comeback and recently, the immense dependence on synthetic products is fall out and humans restore their relation to naturals to gain safety, healthy and security. The main objective of this study was to estimate the correlation between phenolic content and antioxidant activity of methanol extracts of fruits skins and flowers of local varieties of *Ficus carica* originated from two regions of Mascara (west-Algeria): "Ghriss" and "El Bordj". Appreciable rates of polyphenols were noted for all the extracts of fig, the most pronounced total phenolic content were observed for the two type of *Bakor* with green skins fig, 197.47 ± 3.45 and 197.78 ± 2.20 mg GAE g^{-1}, respectively. These amounts can explain the biological activities of the plants' extracts. Positive correlation was proved by this study between different classes of phenolic compounds and the antioxidant capacity of *F. carica* methanolic extracts.
Keywords: Antioxidant activity, *Bakor*, *Ficus carica*, Methanol extracts, Phenolic content.

Introduction

Medicinal and aromatic plants remain a unique and inexhaustible source of useful substances that have very interesting biological properties, which find application in various fields. Plants derived compounds can act as nutraceuticals and as stabilizers for food products. They have also been used for the production of dyes and as crop protection compounds as well as for the manufacture of various health agents (Razzaghi-Abyaneh *et al.*, 2009; Viuda-Martos *et al.*, 2011; Arous *et al.*, 2014; Brahmi *et al.*, 2015). Fruits are one of the essential elements for a balanced diet and they are known for their role in the maintenance of the vital functions of the human organism. The nutritional, dietary and therapeutic value of fruits is unanimously accepted and they are often considered as functional foods (Grigoraş, 2012).

With about 750 species, *Ficus* is among the largest medicinal plant genera (Joseph and Raj, 2011; Ahmad *et al.*, 2013). *Ficus carica* belongs to the family Moraceae, one of the oldest fruits in the world and it is found in tropical and subtropical regions (Vikas and Vijay 2011; Rashid *et al.*, 2014). The fig tree carries three kinds of figs: Early figs (stay on the tree); late, autumn or figs flowers (from August to winter, locally known as *Bakor*); green or winter figs (Paquereau, 2013; Robaine, 2014). Different parts of fig such as leaves, fruit, and root and were used in folk medicine to treat cardiovascular and gastrointestinal disorders, inflammations, colic, diarrhea, indigestion and to heal loss of appetite. In addition, fig was used against respiratory and bronchial problems (Patil *et al.*, 2010). It was also, used as a remedy for visceral obstructive disorders, diabetes, leprosy, and certain skin diseases. In addition, common fig has hypotensives, anthelmintics, antispasmodic and antioxidant effects. (Mousa *et al.*, 1994; Guarrera, 2005). In Algeria, there are two species of fig, one wild, the caprifig, and the other cultivated. The Algerian fig orchard with nearly 5 million trees still remains among the main fruit species in the country and constitutes more than 10% of the national arboreal heritage.

In 1988, it accounted for more than 50% of hardy species other than olive (Chouaki *et al.*, 2006). Oxidative stress known as an imbalance of prooxidant systems and antioxidants ones in favor of the first and involve the production of reactive oxygen species.

The excessive generation of oxygen species like hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) or hydroxyl radical (OH) will have consequences, often heavy for the organism (Pelletier *et al.*, 2003). Oxidative stress is involved in many pathologies as triggering factor or associated with complications. It may be associated with atherosclerosis, hyperoxia, hepatitis, asthma, arthritis, vasospasm, trauma, heart attack, stroke, retinal damage, dermatitis and cancers (Cohen *et al.*, 2000; Packer and Weber, 2001). The aim of the study was to evaluate photochemical contents by determining the total phenolic content (TPC), the total flavonoid content (TFC) and the total anthocyanin content (TAC), and to estimate the correlation which can exist between the phenolic composition of the skins of both figs fruits and figs flowers fruits "*Bakor*" from local varieties of *Ficus*

carica of two regions of Mascara (west-Algeria): "Ghriss" and "El Bordj" and the antioxidant activity.

Materials and Methods

Plant collection

Plants material were collected between July and August 2014 from two regions of Mascara: "Ghriss" and "El Bordj" with 3 varieties from each one (Green, Purple fig and fig flowers *Bakor*). It situated in the southwest of Algeria (latitude: 35°23′47″ N, longitude: 0°08′24″ E). Mascara's climate is classified as warm and temperate (average temperature: Max./Min. (8.3/27.0 °C) and about 487 mm of precipitation falls annually. After their identification, voucher specimens of plants species were deposited at the Laboratory of Biotoxicology, Pharmacognosy and Biological Valuation of Plants, University Dr. Tahar Moulay of Saida, Algeria.

Preparation of plant extract

Six samples of *F. carica* were air-dried and then crushed. Then, 20 mL of pure methanol (99%) was used as solvent to macerate *F. carica* fruits skins (1 g of each) for 72 h under frequent agitation. Extracts filtered and concentrated under reduced pressure. The residues of plants were dissolved using 3 mL of methanol and conserved at 4 °C for future use (Benhammou *et al.,* 2008). Reagents and chemicals used during assays were purchased from Merck (Darmstadt, Germany).

Determination of extraction yields

Extract yield (%) was determined as described as follows (Yihune and Yemata,2019):

$$\text{Extract yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Initial weight of plant powder}} \times 100$$

Determination of total phenolic content (TPC)

Total phenolic content was estimated according to the test described by (El-Haci *et al.,* 2012). Methanolic extract (0.1 mL) was added to 2 mL of sodium carbonate solution (2%). Folin–Ciocalteu reagent (100 µL) was added, 5 min later to the mixture which was, then, incubated for 30min. Absorbance was deduced at 700 nm and TPC was expressed as gallic acid equivalents of dry weight (mg GAE g^{-1}).

Determination of total flavonoids content (TFC)

A volume of 2 mL $AlCl_3$ ethanolic solution (2%) was mixed with 2 mL of each plant sample. The mixture is vigorously stirred and incubated for 10 min at room temperature. The absorbance was measured at 430 nm and TFC was expressed as mg quercetin equivalents of dry weight (Hebi and Eddouks, 2015).

Determination of total anthocyanin content (TAC)

The total anthocyanin content were measured via pH differential test by deducing the absorbance of the mixture at two wavelengths 510 and 700 nm using two buffer systems at pH 1,0 (hydrochloric acid, 0.2 M) and at pH 4,5 (sodium acetate, 1 M).

$$A = (A510\text{-}A700)_{pH1,0} - (A510\text{-}A700)_{pH4,5}$$
$$TAL = [(A \times MW \times DF) / MA] \times 100$$

A: absorbance; MW: molar mass; DF: dilution factor; MA: molar absorption.
TAL was expressed as mg cyanidin-3-glucoside equivalent of dry weight (Lako *et al.,* 2007; Ercisli *et al.,* 2012). All phytochemical tests were carried out in triplicate.

Quantitation of total antioxidant activity (TAA)

Total antioxidant activity of extracts was estimated via the phosphomolybdenum assay described by (Prieto *et al.,* 1999). Plant extract (0.3 mL) was added to 3 mL of reagents mixture (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). Then, the mixture was incubated at 95 °C, 90 min. Absorbance was measured at 695 nm and compared with control that contained 0.3 mL methanol mixed to 3 mL of the reagents mixture cited above incubated in the same conditions. TAA was expressed as mg ascorbic acid equivalents per g of dry weight (mg AAE/ g). A calibration curve was performed by ascorbic acid.

DPPH scavenging assay

The hydrogen atom donation capacity of phytochemical components in *F. carica* methanolic extracts was measured on the basis of the scavenging of DPPH free radical (2,2-diphenyl-1-picrylhydrazil) **(**Sanchez-Moreno *et al.,* 1998). Methanol solution (1 mL) and DPPH (100 µM) were mixed to 0.1 mL of various methanolic extracts. After a 20 min incubation period at room temperature, the absorbance was deduced at 517 nm with comparison to a blank composed of 1 mL of DPPH methanol solution and 0.1 mL methanol (Doukani *et al.,* 2014). The decrease of absorbance was read and inhibition percentage (IP) was then calculated as follows:

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

Where: $\mathbf{At_0}$**:** Absorbance of blank after 20 min. $\mathbf{At_{20}}$**:** Absorbance of samples after 20 min.
The concentration of extract providing 50% inhibition (EC50) of DPPH was measured by linear regression using the graph which related extract concentrations and inhibition percentage. A methanolic solution made of ascorbic acid was served as positive control (Fabri *et al.,* 2009). As cited by (Ladoh Yemeda *et al.,* 2014), antiradical ability (PA) is inversely proportional to EC50:

PA = 1 / EC50

Statistical analysis

All measurements of antioxidant ability were performed three times. Data were expressed as means ±standard deviation each means used were deemed to be significant at $P \leq 0.05$. The difference between the tested varieties of the two regions were analyzed by the two-way ANOVA test. Excel program was used to determine the correlation coefficient of antioxidant activity.

Results and Discussion

Extract yields and phenolic contents estimation

The phenolic contents and total antioxidant activity of *F. carica* methanol extracts are summarized in Fig. 1 and 2. Relation between Radical scavenging activity and phenols content of various extracts is illustrated in Fig. 3, 8. DPPH EC50 of *F. carica* methanol extracts is given in Fig. 9. The linear correlation coefficient between total antioxidant activity and total phenolic content was r = 0.266 and it was r = 0.979 between total antioxidant activity and total anthocyanin content. The correlation coefficient was r = 0.06 between total antioxidant activity and total flavonoid content. The correlation coefficient between DPPH EC50 and total anthocyanin content was r = 0.303 and it was r = 0.277 with total flavonoid content. However, the correlation was r = -0.824 between DPPH EC50 and total phenolic content.

The analysis of these values showed that the best yield has been recorded for the varietyF1 (bakor of Ghriss) 28% followed by F4 (green fig of El Bordj) 26.7%, F3 (green fig of Ghriss) 25.3%, F5 (purple fig of Ghriss) 24.2%, F6 (purple fig of El Bordj) 23.9%, F2 (bakor of El Bordj) 23%. Polar solvents were more effective at extracting phenolic compounds that are potent antioxidants. Isolation of plant compounds is widely dependent on the nature of the chosen solvent during the extraction process. Quantitatively, methanol is the best solvent used to extract material samples than any of the other solvents by its greatest amounts resulted (Masoko *et al.,* 2007). Furthermore, the nature of solvent used for extraction can be a crucial parameter as the most important extraction yields for *Haloxylon scoparium* were obtained using polar solvents such as water followed by methanol (Messaoudi *et al.,* 2019). Similarly, yields of dry extracts of leaves of *F. carica* obtained by Nicotra *et al.,* (2010), by hydroalcoholic mixtures with ethanol (50%) and (70%) were 57.50% and 56.80% respectively. A probable effect of extraction time of more than 72 hours and using the pure methanol solvent (99%) on the important yields remains an undisclosed proposition. Levels of phenolic and flavonoid compounds of herbal samples changed significantly from an area to another, which can be explained by the nonuniformities of the temperature, solar lighting, soil type, climate and humidity of each study area (Messaoudi *et al.,* 2019). In this study of fig skin, significant amounts of polyphenols were found in all varieties, the most pronounced were observed for the two type of bakor (F1, F2) with green skins fig, 197.47 ± 3.45 and 197.78 ± 2.20 mg gallic acid equivalents g^{-1} of dry weight, respectively. Estimates of total flavonoid content in the skin of tested varieties of *F. carica* were in the following order: F6>F3>F4>F1>F2>F5, in which, the purple fig skin F6 dominated the other type of fig with a composition of 14.78 ± 2.12 mg quercetin equivalent g^{-1} dry weight. These high levels of total phenolic content and total flavonoid content are explainable as phenolic compounds, particularly flavonoids function as UV filters against deleterious actions of UV radiation, preserving then cellular structures like chloroplasts (Oliveira *et al.,* 2009). These values indicate that the skin of *F. carica* is a plant part bearing appreciable levels of phenolic compounds. Figs, whether fresh or dried, contain relatively high amounts of polyphenols (Patil Vikas *et al.,* 2010). Our results are in agreement with earlier findings (Ercisli *et al.,* 2012; Çaliskan and Polat, 2011), who have reported higher total phenolic content levels in the skin of *F. carica* genotypes colored with black, dark purple and violet color compared to genotypes with yellow-green, light green, and light purple-skinned that contained a lower amount of total phenolic content. The values detected ranged between 24 and 237 mg gallic acid equivalents 100 g^{-1} of fresh weight (FW) and varied in other researches from 28.6 to 211.9 mg gallic acid equivalents 100 g^{-1} of fresh weight. Park *et al.* (2013) have demonstrated that ethyl acetate extract from the fig tree branches contained the highest level of total phenolic content which was 611, 47 mg gallic acid equivalents g^{-1} of dry weight. Further results recorded by El-Shobaki *et al.* (2010) and Saad *et al.* (2012) have suggested the impact of the stage of maturation on total phenolic content of *F. carica*, which were 36 before maturity, 40 at maturity and 49 mg gallic acid equivalents 100 g^{-1} at an advanced stage of maturity. According to the statistical analysis, there are non-significant differences between the extracts of Ghriss and El Bordj ($P > 0.05$).

All fig varieties showed an exceptional richness in phenolic compounds proven by the appreciable levels measured by the various tests and assays carried out but also by the well-known positive impact of such molecules on health. The total antioxidant activity deduced was in the following order: (F5 = F4 = F3) > F6> F2> F1. It appears that the figs F5 (purple), F4 and F3 (green) exhibited almost the same most potent AAT with 38.16 mg ascorbic acid equivalents g^{-1}. A recent work has reported that among all the fruits and vegetables common in the diet, figs especially those with blue or dark red colors have the highest

antioxidant capacity (Çaliskan and Polat, 2011). According to El-Sayed Saleh (2009), the antioxidant capacity of phenolic molecules has been attributed to its oxidative-reducing abilities that enable them to execute a reducing action or by scavenging oxygen or generating hydrogen. Thus, *F. carica* endowed with more antioxidant ability (Joseph and Raj, 2011). Chawla *et al.* (2012) reported that dried fruits of *F. carica* have significant antioxidant properties. Besides, after their human consumption, dried figs act *in vitro* as antioxidants. It was noticed that the existence of an average correlation between total phenolic content and total antioxidant activity(r = 0.515), is weak with total flavonoid content (r = 0.248) whose antioxidant activity does not exceed 24.8% of phenolic compounds. Conversely, the reducing activity is strongly correlated with the total anthocyanin content with r = 0.989 resulting from the action of anthocyanin 98.9%. Many research groups including (Djeridane *et al.*, 2006; Wong *et al.*, 2006; Wojdylo *et al.*, 2007; Tawaha *et al.*, 2007; Turkmen *et al.*, 2007), have declared that a positive correlation related antioxidant activity with total phenolic content. The DPPH test deduces the EC50, the concentration of an extract that produces 50% inhibition of DPPH activity, the smaller its value, the more this extract is a powerful antioxidant (Scherer *et al.*, 2009; Kadri *et al.*, 2011; Zhang *et al.*, 2015). It can be concluded from correlation figures that non-significant differences ($P \leq 0.05$) can be found in the antioxidant effect of the tested plants' extracts. Through this study, the early fig (fig flowers or *bakor*) has contained a high amount of polyphenols and presented an important antioxidant activity and need to be more explored to elucidate any other possible biological properties. In addition, the methanol is more effective to extract phenolic components and the solvent type has proven to be an important and decisive factor of extraction. Plant extracts have high potential as antioxidant agents, thus, they can be used in the food and therapeutic factories. Although they have not been completely investigated, the great potential of medicinal plants for food and pharmaceutic purposes has been revealed by the cited results as well as literature data. So, future studies will have to be conducted to discover new bioactive agents.

Conclusion

F. carica methanolic extracts showed an exceptional richness in phenolic substances, supported by the considerable levels estimated by the multiple assays but also by the well-known positive impact of such compounds on human health. Furthermore, the results prove that methanolic extracts obtained from *F. carica* displayed a strong antioxidant ability. It can, therefore, be inferred that those extracts could be useful as a natural antioxidant agents. Moreover, the findings of this study could be valuable for future studies to identify and purify bioactive compounds responsible for such activity, then elucidate their exact role for possible applications for food preservation purposes. Algerian flora is varied and highly rich, thus it can constitute an important source of very interesting plant molecules that can be used in various fields.

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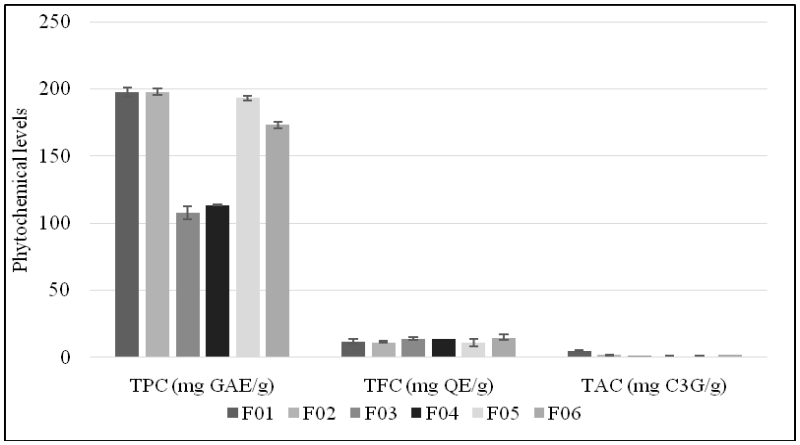

Fig. 1: Phytochemical screening of *Ficus carica* methanol extracts.
F_{01}: Bakor (fig flowers) of Ghriss; $\mathbf{F_{02}}$: Bakor (fig flowers) of El Bordj; $\mathbf{F_{03}}$: Green Fig of Ghriss; $\mathbf{F_{04}}$: Green Fig of El Bordj; $\mathbf{F_{05}}$: Purple Fig of Ghriss; $\mathbf{F_{06}}$: Purple Fig of El Bordj.
TPC: Total phenolic content. **TFC:** Total flavonoid content. **TAC:** Total anthocyanin content. **mg GAE g^{-1}:** acid gallic equivalents. **mg QE g^{-1}:** quercetin equivalents. **mg C3G g^{-1}:** cyanidin-3-glucoside equivalents.

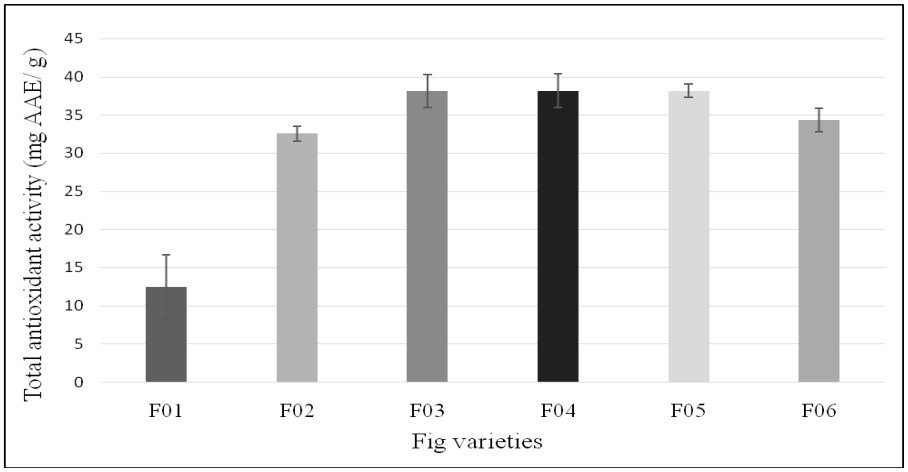

Fig. 2: Total antioxidant activity of *Ficus carica* methanol extracts.
F01: Bakor (fig flowers) of Ghriss; **F02**: Bakor (fig flowers) of El Bordj; **F03**: Green Fig of Ghriss; **F04**: Green Fig of El Bordj; **F05**: Purple Fig of Ghriss; **F06**: Purple Fig of El Bordj.

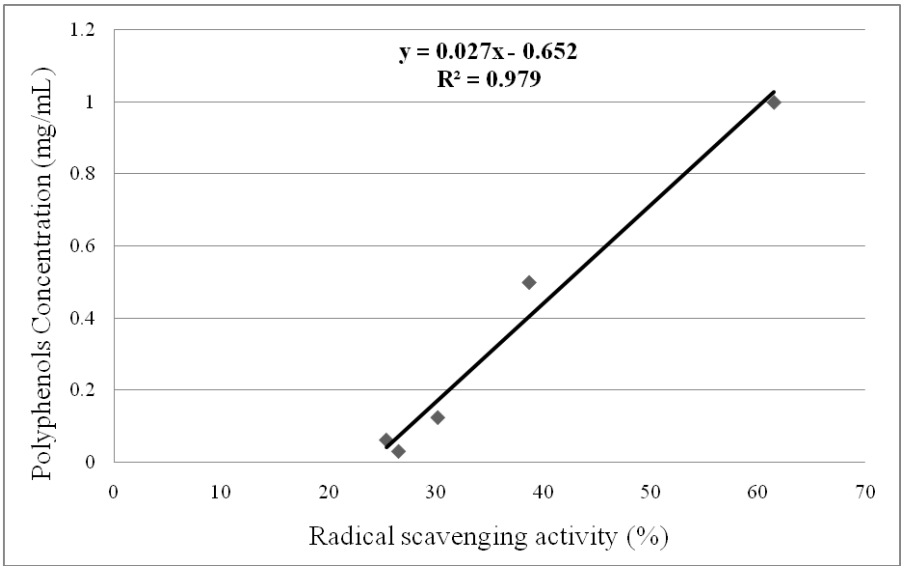

Fig. 3: Radical scavenging activity (DPPH %) of Bakor (fig flowers) of Ghriss (**F01**).

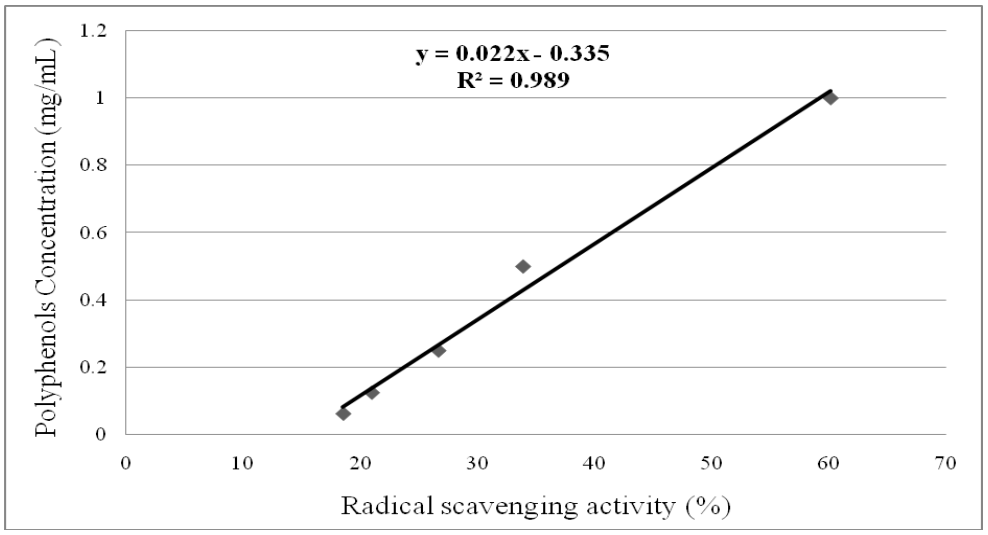

Fig. 4: Radical scavenging activity (DPPH %) of *Bakor* (fig flowers) of El Bordj (**F02**).

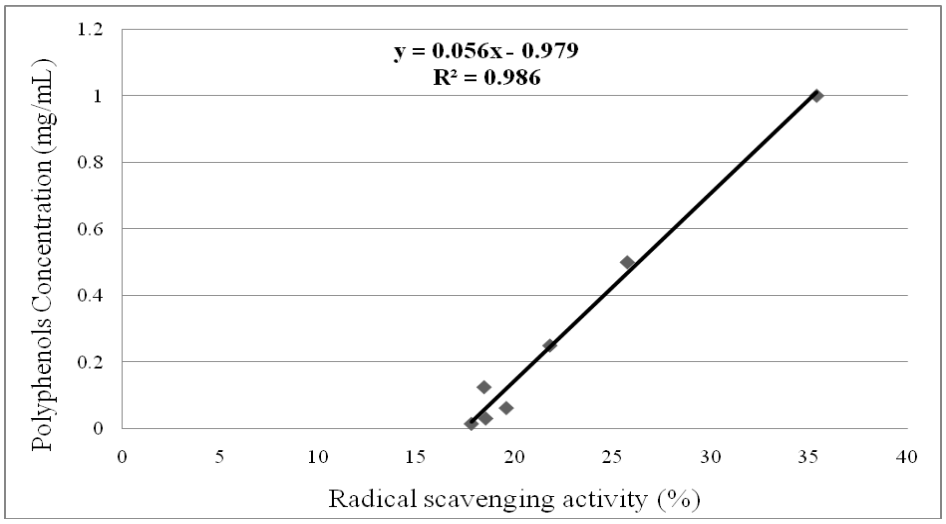

Fig. 5: Radical scavenging activity (DPPH %) of green fig of Ghriss (**F_{03}**).

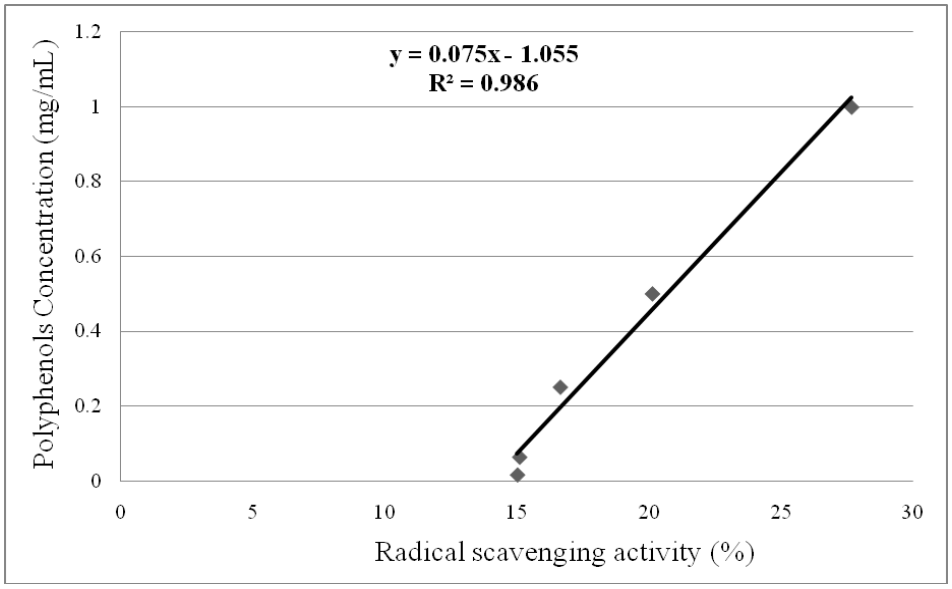

Fig. 6: Radical scavenging activity (DPPH %) of Green Fig of El Bordj (**F_{04}**).

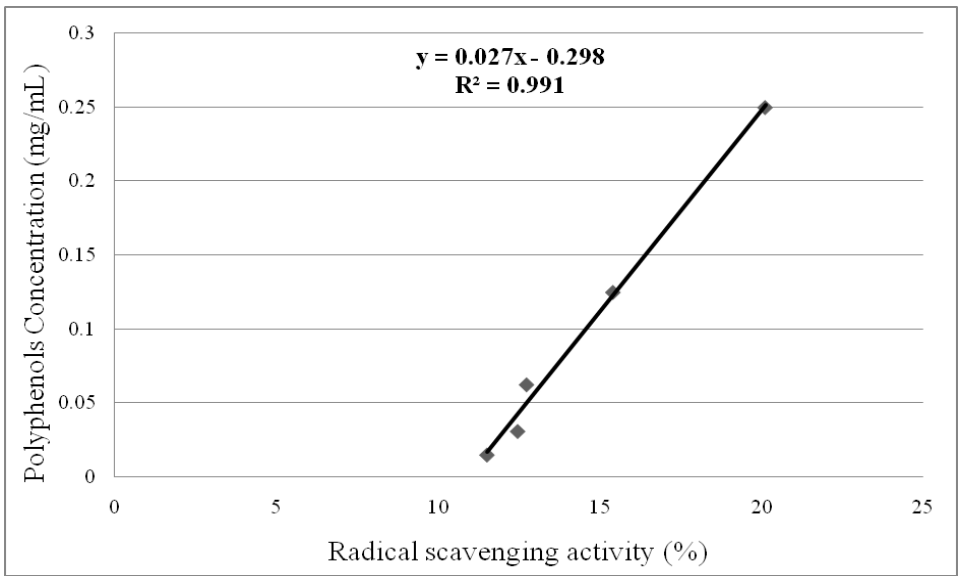

Fig. 7: Radical scavenging activity (DPPH %) of purple fig of Ghriss (**F_{05}**).

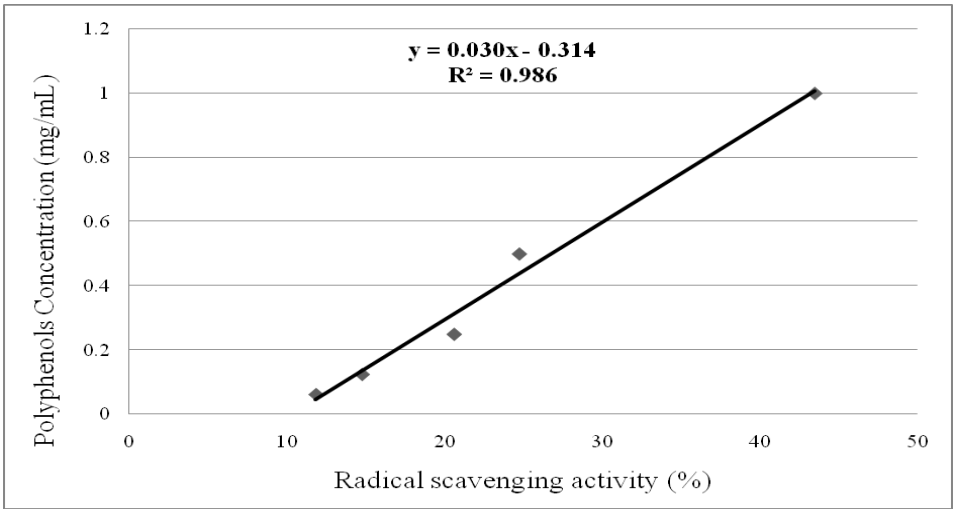

Fig. 8: Radical scavenging activity (DPPH %) of purple fig of El Bordj (**F_{06}**).

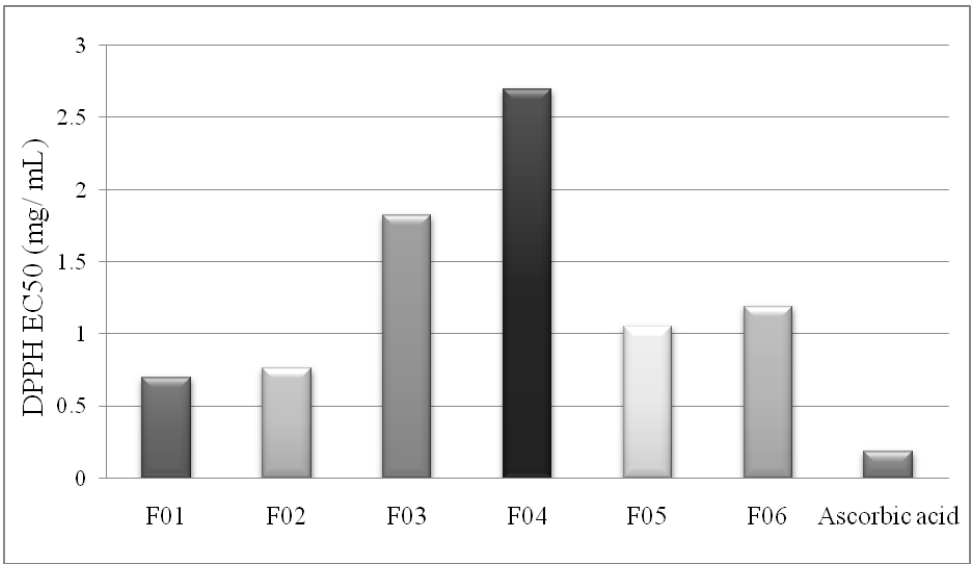

Fig. 9: DPPH EC50 of *Ficus carica* methanol extracts.
F_{01}: Bakor (fig flowers) of Ghriss; **F_{02}**: Bakor (fig flowers) of El Bordj; **F_{03}**: Green Fig of Ghriss; **F_{04}**: Green Fig of El Bordj; **F_{05}**: Purple Fig of Ghriss; **F_{06}**: Purple Fig of El Bordj.

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