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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THREE SAMPLES OF DRIED FIGS (*FICUS CARICA L.*) FROM THE REGION OF MASCARA (WESTERN ALGERIA)

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ABSTRACT

Ficus carica L. have always been known by their power to cure various diseases. This study aims to evaluate the antioxidant activity of twelve different extracts of three samples of dried figs. First, the extracts were tested for their polyphenolic, flavonoid and tannin contents by the Folin–Ciocalteu, the aluminium trichloride and vanillin methods and high-performance liquid chromatography (HPLC) for more characterisation. The antioxidant activity was performed by using thre different methods; the 2,2-diphenyl-2-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAC). The results showed that the methanolic extract of Sidi Bendjebbar sample presented a higher TPC value (458 mg GAE /g) while the acetonic and aqueous extracts of El-Keurt presented a higher values of TFC and CTC. In other hand, the methanol extract of El-Keurt sample exhibited the highest antioxidant capacity with an IC_{50} of 0.078 mg/ml. The antimicrobial activity of the extracts against various Gram positive and Gram negative bacteria was screened by the inhibition zone using the disc and diffusion assay, minimal inhibition concentration (MIC) by micro-well method allonwing to calculate the fractional inhibitory concentration (FIC). The ethanolic extracts of the two samples El-Keurt and Sidi Bendjebbar were the most effective extracts being able to inhibit the growth of the majority of the strains tested. Our study supported the use of these fruit as supplements for nutrient deficiencies and for combating diseases associated with oxydative damage or some microbial infection for better drug alternatives.

Keywords: *Ficus carica*, polyphenols, antioxidant activity, antimicrobial activity, dried figs, MIC

INTRODUCTION

The fig tree (*Ficus carica); Moraceae* family*;* is the second emblem in the Mediterranean basin where it has been cultivated for millennia. The dried fruits of *F. carica* have been reported as an important source of nutritional elements as vitamins, minerals organic acids and phenols (Mawa et *al*., 2013). Previous studies have been performed on polyphenolic extracts of *F. carica* and reported numerous bioactive compounds i.e. phytosterols, organic acids, anthocyanin, triterpenoids, volatile compounds and coumarins (Oliveira et *al*., 2009, Nasar et *al*., 2014).
This Fruits are traditionally used to cure many diseases and as a stimulant, laxative, emollient, resolutive cough suppressant, emmenagogue (Guarrera, 2003) and for constipation, hemorrhoids and hypercholesterolemia (Cansaran and Kaya, 2010). These fruits have been able to increase antioxidant capacity in plasma not to mention their benefits on various disorders such as inflammatory, cardiovascular disorders, gastro-intestinal, respiratory, ulcerative diseases, and cancers (Vinson et *al*., 2005; Jasmine et *al*., 2014). Solomon and colleagues (2006) reported that *F. carica* fruits contains produces the highest antioxidant effect.
Biomolecules have always interested scientists working on infectious diseases highlighting plants with antimicrobial activity (Aswar et al., 2008). Infectious diseases continue to represent a critical problem to human health incresed using of antibiotics and vaccination programs (Adeshina et *al*., 2010). At present, many bacteria pose a huge problem because of the multidrug resistance that may have developed to the antimicrobial agents used against infections, making the discovery of new antimicrobial substances an undesirable action (Chanda and Kaneria, 2011).
Viewing the alternative properties of *F. carica*, our study deals with different extracts of dried figs grown in three different regions of Mascara (El-Keurt, Ain Fares and Sidi Bendjebbar) to investigate their phytochemical compounds and antioxidant activities to determinate finally the correlation between these activities to investigate their antibacterial activity against 14 bacteria.

MATERIALS AND METHODS

Plant samples

Dried figs were collected from local markets in the three regions (El-Keurt, Ain Farés and Sidi Bendjebbar) of Mascara during the month of December 2014. The varieties were confirmed by the Technical Institute of Mascara Fruit Trees (ITAF) after being selected according to market availability, the most frequent consumption and the altitude of the growing area.

Preparation of the extract

The extracts were prepared according to the modified method described by Jasmin and colleagues (2014). Aqueous and organic extracts were prepared from 50 g of the pulp macerated in 200 ml of solvent (distilled water, 80% methanol, 70% ethanol and 50% acetone) at room temperature and away from light for 24 hours, under agitation. Then, the mixture was filtered and concentrated with rotavapor at 40°C under vacum to obtain a dry extract permitting the calculation of the yield of each sample.

Phytochemical Screening

The qualitative characterization of the aqueous, methanolic, ethanolic and acetonic extracts was carried out according to Evans 1996 by chemical techniques and thin layer chromatography tests.

Determination of total phenolics content

The Folin–Ciocalteu method has helped to determine the TPC of *Ficus carica* extracts following to Singleton et *al*. (1999) with some modification. 20 µl of each

extract was diluted in 1.58 ml of distilled water. An aliquot of the solution was added to 100 µl of Folin-Ciocalteu reagent diluted in distilled H_2O (v / v) before adding 300 µl of sodium carbonate 7.5%. After 2 hours of incubation, protected from light, read the absorbance from the UV-visible spectrophotometer at 760 nm. The blank is represented by methanol added to the Folin-Ciocalteu, distilled water and sodium carbonate. All measurements are repeated three times using gallic acid as a standard. The results were expressed as mg gallic acid equivalents (GAE)/100 g dried fruit.

Determination of total flavonoids content

Quantification of flavonoids (TFC) was carried out by a spectrophotometric method adapted Zhishen et *al.* (1999). 500 µl taken from different concentrations of methanolic extract and catechin solution diluted in methanol were added to 1500 µl of distilled water and then mixed with 150 µl of sodium nitrite ($NaNO_2$) at 5% and 150 µl and 10% aluminum trichloride ($AlCl_3$). 500 µl of sodium hydroxide (1 M NaOH) are added after incubation for 5-6 min. Absorbance was measured at 510 nm against white. The total flavonoid content of the extracts was expressed in milligrams (mg) of catechin equivalent per gram (g) weight of dry matter (EC) /g).

Determination condensed tannins content

The amounts of condensed tannins (CTC) are determined by the vanillin method (Julkunen-Titto, 1985). The vanillin solution was prepared by mixing in equal volume: 8% HCl (v / v), 37% methanol (v / v) and 4% vanillin in methanol (w / v). The mixture was maintained at 30 ° C before assay (Ba et *al*., 2010). 50 µl of each extract is mixed with 1500 µl of vanillin / methanol solution and added to 750 µl of concentrated hydrochloric acid (HCl) to be incubated for 20 min. Absorbance was measured at 550 nm against a blank consisting of a mixture of methanol (37%) and HCl (8%) in equal volumes (Mahmoudi et *al*., 2013). The tannin concentration s determined in mg of catechin equivalent per gram (g) of the dry matter weight (EC) / g).

Determination of antioxidant activities

Total antioxidant activity

Total antioxidant capacity (TAC) was tested by the phospho-molybdenum method Prieto et *al*. (1999). A series of solutions containing 0.3 ml of the various extracts is added to 1.2 ml of a reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 M ammonium molybdate). The absorbance was measured at 695 nm (Puoci et *al*., 2011) after having incubated the mixture for 150 min at 95 ° C against the blank consisting of 0.3 ml of methanol and 3 ml of the previously prepared reagent. This activity was expressed in milligrams equivalents of ascorbic acid per gram of dry matter (mg AAE / g DM). The experience is repeated in triplicate (Isaac Kingsley Amponsah, 2012).

DPPH radical-scavenging activity

The determination of antioxidant activity by the DPPH (diphenyl 2.2 picrylhydrazyl 1) was determined by the method of Brand-Williams et *al*. (1995). Before starting, the DPPH solution using 0.0197g of DPPH to dissolve in 100 ml absolute methanol was prepared to have a solution of 0.5 mM. For all extracts, dilutions were prepared in absolute methanol in order of micrograms per ml. The solution of the test extract previously diluted with a Tris buffer solution (0.1 M, pH = 7.4) was added to the DPPH solution. After agitation, the tubes are placed in the dark for 30 minutes. The absorbance was measured at 517 nm against the blank which was composed of 1 ml of methanolic DPPH solution (0.3 mM) and 1 ml of the Tris solution. The positive control was represented by ascorbic acid, to calculate the EC_{50} effective concentration that reduces the initial concentration of 50% DPPH, the results were expressed as percent inhibition according to Wang and Mazza (2002):

$$\%Inhibition = \{(Abs\ contrôle - Abs\ test) / Abs\ contrôle\} \times 100$$

Ferric reducing antioxidant power (FRAP)

The iron-reducing activity of our samples was determined according to the method described by Oyaizu (1986). In a tube was mixed a volume of extract at different concentrations, 2.5 ml of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 ml of potassium ferricyanide solution $K_3Fe(CN)_6$ to 1% before incubating at 50 ° C / 20 minutes. 2.5 ml of 10% trichloroacetic acid was added to perform centrifugation at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant was reacted with 2.5 ml of distilled water and 0.5 ml of a frechely prepared ferric chloride solution at 0.1%. The absorbance was measured at 700 nm using a blank containing the constituents mentioned above except the extract which was replaced by distilled water. An increase in the absorbance corresponds to an increase in the reducing power of the extracts tested (Hubert, 2006).

HPLC-DAD for phenolic profile determination

The extracts were subjected to HPLC analysis on an HPLC system Agilent 1200 (USA) type with a diode array detector. Column Hichrom C18 (4.6 mm x 250 mm, particle size of 5 microns). This device includes a column temperature of 40 ° C allowing an injection volume of 10 µl to pass. The solvent system was a gradient of water-formic acide (0.5%) (A) and methanol (B). The gradient employed was: starting with 95% (A), from 95% (A) to 60% for 30 min, from 60% (A) to 35% for 15 min at a flow rate of 1.0 ml/min. The detection spectra of the polyphenols were noted at 280 nm and 320nm.

Determination of the antibacterial activity

Test microorganisms

Five gram positive bacteria *Listeria innocua, Bacillus subtilis, Clostridium perfringens, Enterococcus faecalis* and *Staphylococcus aureus* and nine gram-negative bacteria : *Vibrio cholerae, Pseudomonas aeruginosa, Escherichia coli, Enterobacter sakazakii, Enterobacter cloacae, Proteus mirabilis, Citrobacter freundii, Klebsiella oxytoca* and *Serratia odorifera* were tested in the screning. The microorganisms were isolated from the laboratories of microbiology of Yessad Khaled and Meslem Tayeb hospitals (Mascara, Algeria) and identify using the MALDI-TOF-SM except *Listeria innocua* and *Staphylococcus aureus* were obtained from Department of Food Technology and Nutrition, Catholic University of Murcia (Spain).

Evaluation of antibacterial activity and Minimum Inhibitory Concentration (MIC)

In vitro antimicrobial activity of the twelve extracts of *Ficus carica* dry fruits was studied against 14 pathogenic microbial strains by using the disc diffusion method according to Bauer et *al*. (1966). For this study, Gentamycin discs were used as positive and DMSO as negative control. Each standardized inoculum (0.5 McFarland) was melted with Muller Hinton agar cooled to 48°C and poured into sterile Petri dishes (Chanda and Kaneria, 2011). Blank discs were impregnated with the extracts already dissolved in pure DMSO at concentrations of 80, 40, 20 and 10 mg / ml in order to return them to the surface of the previously inoculated agar of each microorganism. Antimicrobial activity was recorded by measuring the clear inhibition zones around each disc after an incubation of 37 ° C for 24 hours (Aimahy et *al*., 2003).
The second method to evaluate the antibacterial activity was by using 96 Well microliter plates (Mitscher et al., 1972). A dilution series was performed in the wells ranging from 150 µg/ml to 1.17 µg (Lazreg Aref et *al*., 2010).

Antimicrobial effects of combined extracts

This technique allowed to study the combination of 12 extracts in order to have the most relevant antimicrobial activity using 2 different methods: microplates and diffusion on agar medium. The interpretation of the results was based on the growth of bacteria at different concentrations of combined extracts to conclude the synergistic effect, cumulative, indifferent and antagonistic according to the FIC values: FIC ≤0. 5, FIC = 0.5-1, FIC = 1-4 and FIC ≤4 respectively (Climo et *al*., 1999).

Statistical analysis

The data were analyzed statistically using one-way analysis of variance (STAVIEW version 5.0, Abacus Concepts, Berkeley, CA) and Student's t-test. The results are given as arithmetic mean ± SEM. The correlation between antioxidant capacity and polyphenol content was determined by the Pearson correlation (R^2 value).

RESULTS

Physicochemical Analysis

Investigations on the phytochemical screening of *Ficus carica* fruits of the three samples indicated on table 1, the presence of flavonoids, tannins, coumarines and alkaloids, total phenols coumpond and anthocyanins in faint quantity, while saponins, Steroids and triterpenoids are totaly absent. Previous studies realized by Vaya et Mahmood (2006), Teixeira et *al*. (2006) showed that the aqueous extract of *Ficus carica* contains alkaloids, flavonoids and coumarins. *Ficus exasperata* includes alkaloids and tannins without any traces of saponosides or sterols (Engwa et *al*., 2015).

Table 1 Results of phytochemical screening of *Ficus carica* samples

	El-Keurt	Ain Farès	Sidi Bendjebbar
Phenols	±	±	±
Flavonoids	+	+	+
Tannins	+	±	±
Coumarines	+	+	+
Alkaloids	+	+	+
Saponins	-	-	-
Anthocyanins	+	±	-
Steroids and Triterpenoids	-	-	-

+ Present, ± traces, - absent.

Total phenolic compound

The TPC was determined by employing Folin-Ciocalteau reagent and was expressed as mg of gallic acid per 100g of dry fruit. The TPC of the three samples varied from 78 to 458 mg GAE/100g of DM. The highest value was conferring to the methanolic extract of Sidi Bendjebbar sample and the lowest is the aqueous extract of Ain Farès (Fig.1). According to the work of Doha and Al-Okbi (2008), figs have the lowest polyphenol content with 920 mg GAE / 100 g of DM compared to other plants eg rosewood that contains more than 8643 mg GAE / 100 gr MS. The total polyphenol content studied by Bey and Louaileche (2015) ranges from 482.62 mg GAE / 100gr MS (Aberkane) to 644.11 mg GAE / 100gr MS (Taghanimt) to reveal that the black fruits of *Ficus carica L.* are richer in polyphenols than the white samples. These values are higher than ours, since our values have not exceeded 458±0.42 mg GAE/ 100 g DM.

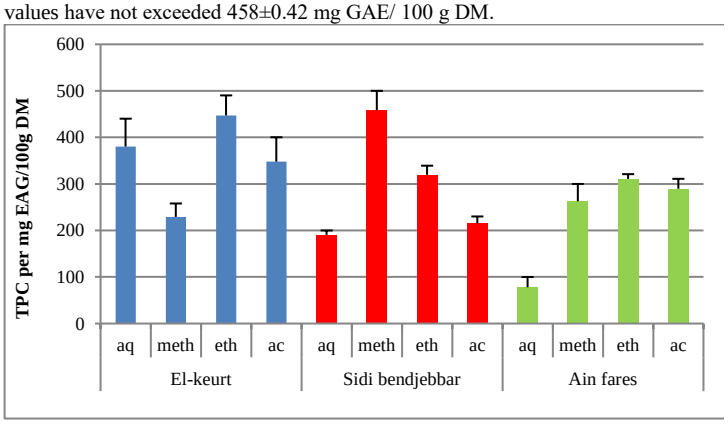

Figure1 Total polyphenol content (TPC) of Ficus carica extracts (mg GAE / 100 g DM) ***aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.***

Total flavonoid compound

In this study, the amount of total flavonoids was expressed using the $AlCl_3$ reagent and catechin as standard (R^2= 0,9937). The total flavonoids varied from 38.8±0.012 mg CE/100 g DM as lowest value (methanolic extract of Ain Farès) to 228.22 ± 0.27 CE/100 g DM as the highest value (acetonic extract of El-Keurt sample). The total flavonoids that Lamien-Meda et *al.* (2008) were able to detect in the *Ficus sycomorus* species of Burkina Faso had a value of 24.15 ± 1.81 mg QE / 100g of fruit for the methanol extract and 33.15 ± 1.79 mg QE / 100g of fruit for the acetonic extract, which fits perfectly with our work where the acetonic extract of the three samples allowed to extract the highest content of flavonoids (fig.02). *Ficus bengalensis* had the highest level of flananols with more than 3 mg QE / g of DM compared to the other used plants and a flavonoid level above our three samples with 5 mg QE / g of dry extract (Sharma et *al*., 2009).

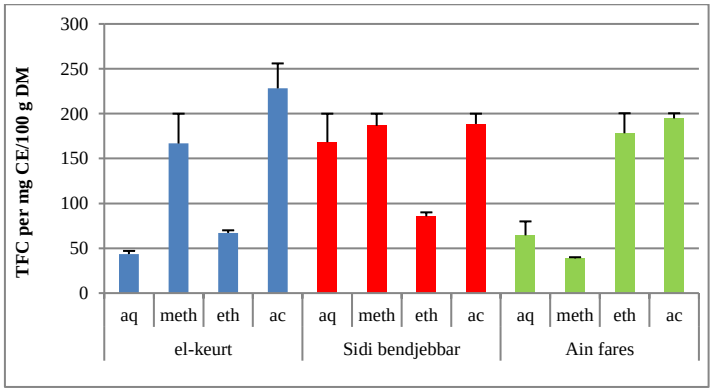

Figure 2 Total flavonoids content (TFC) of *Ficus carica* extracts (mg CE / 100 g DM) ***aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.***

Condensed tannins content

As depicted in Fig. 3, the highest total tannin content and the lowest value were observed in El-Keurt sample with 254,1 ± 0.43 mg CE/100 g DM for the aqueous extract and 7.05 ± 0.3 mg CE/100 g DM for the ethanolic extract. If we compare these results with other studies, we find that Debib et *al*. (2013) had a tannin content between 10 and 194 mg GAE / 100 g where the highest value was observed for the methanol extract of the two samples with 160 to 194 mg GAE / 100 g and the smallest is for extracts macerated with petroleum ether in contrast to our extracts since the lowest levels were in methanol extracts with a max of 122.35 ± 2.16 mg GAE /100 g DM.

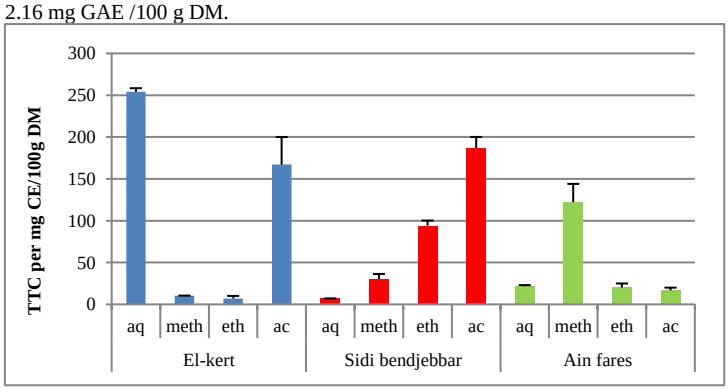

Figure 3 total tannin content (TTC) of Ficus carica extracts (mg CE / 100 g DM). ***aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.***

Antioxidant activities

The antioxidant capacities of the three varieties were determined using free radical scavenging capacity (DPPH), ferric reducing antioxidant power (FRAP) and total antioxidant activity (TAC).

Total antioxidant activity

This assay is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Pioci et *al*., 2011). Total antioxidant activity of all extracts was expressed as mg equivalent of gallic acid per g of dry mater by using different concentrations, a calibration curve was recorded, and the correlation coefficient (R^2 = 0.9989). According to the figure n°4, the antioxidant capacity for *Ficus carica* fruits ranged from 50.5 ± 0.12 to 98.8±0.27 mg AAC/g DM. It was observed that the highest values of this activity are obtained for the ethanolic extract of Ain Farès sample. In second position we find the same extract of Sidi Bendjebbar sample with 95.9 ± 0.2 mg AAC/g DM but the last value was detected in Sidi Bendjebbar sample in comparison with ascorbic acid 99.8 ± 0.13 mg AAC/g DM.

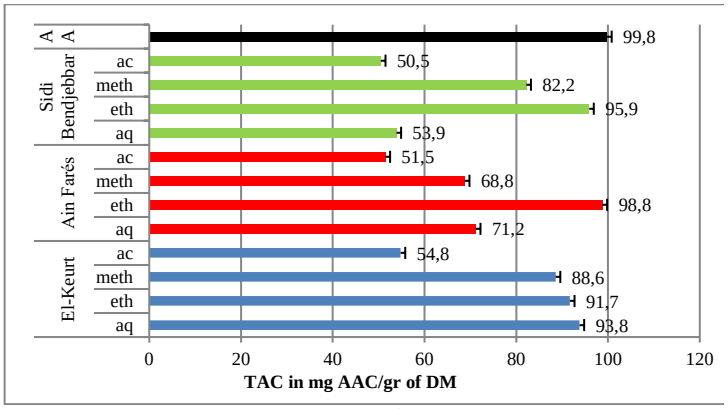

Figure 4 Total antioxidant capacity (TAC) of *Ficus carica* extracts (mg AAC / g DM). ***aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.***

Caliskan and *al*. (2011) worked on different adhesions of *Ficus carica L*. They detected that TAC ranged from 3.9 to 16.1 mmol Fe^{2+} / Kg FW specifying that the adhesion "Siyah 5", which is characterized by a dark black fruit, contained the greatest amount of TAC among the 50 accessions tested. According to Konyaloglu and *coll*. (2005) works, the aqueous extract of the dried figs revealed the highest total antioxidant activity with 23,507 ±1,154 mM α-tocopherol acetate / g of DM followed by methanol and ethanol extract with more than 17 and 14 mM α-tocopherol acetate / g of DM which is not suitable for our work since the ethanolic extracts are the most effective.

DPPH radical scavenging activity

In figure 5, ethanolic extract of El-Keurt sample chelated more than 88.1±0.03% of the DPPH radical with the lowest IC_{50} = 0.0782 mg/g DM compared to the positive control with IC_{50} equal to 0.006 mg/g DM. This high percent of inhibition is explain by his high percent of total phenolic coupounds (the second position) and followed by methanolic extract of the same sample being the most effective sample which had an IC_{50} =0.1016 mg/g DM and a pourcent of inhibition 84.6 ± 0.04 %. The lowest percent was detected in ethanolic extract of the sample Sidi Bendjebbar with a $1/IC_{50}$ closed to 2,458 mg/g DM.

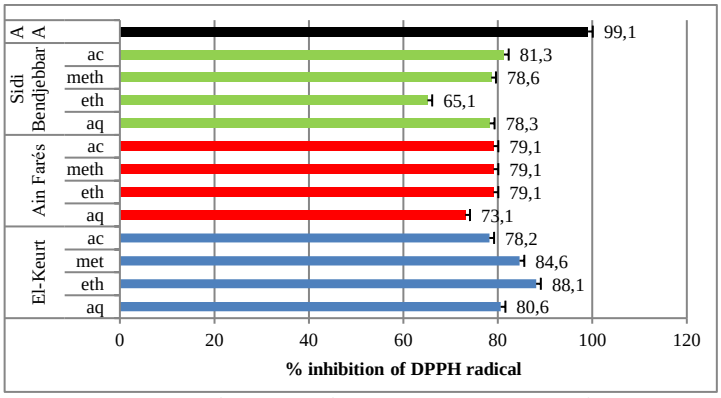

Figure 5 *Pourcentage of inhibition of DPPH radical scavenging* of *Ficus carica* extracts. *Acid ascorbic (AA) was used as standart.*
aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.

In the studies of Konan et *al*.,(2014), it was found that the antioxidant activity value (IC_{50}) of *Ficus dicranostyla* was more than 38.4 µg/ml. The antioxidant capacity evaluated by DPPH radical scavenging ranged from 3.19 to 87.09 mg AAC/g of fw for MeOH extracts and from 4.99 to 107.29 mg AAC/g of fw for the acetonic extracts (Lamien-Meda et *al*., 2008). In other work, the antioxidant activity of dried figs grown in Turkey was 1.087 ± 11 mg AAC / 100 g DM and that of fresh figs grown in Japan was 2.524 ± 37 mg AAC / 100 g DM (Ishiwata et *al*., 2004). These results are in agreement with our work since we had values ranging from 2.458 to 12.787 mg/g DM.

FRAP radical scavenging method

In the FRAP method, the formation of an intense blue color where the intensity is relative to the amount of antioxidant reductants in the sample is mainly due to the reduction of ferric tripyridyltriazine to a ferrous complex detected at a wavelength of 593 nm.. In relation to the solvent used, highest percent of inhibition of FRAP complex was found in acetonic extract of Ain Farès sample with IC_{50} equal to 1,179 mg/g DM followed by the aqueous extract of El-Keurt sample with 1.241 mg/g DM in total agreement with the total antioxidant activity. The aqueous extract of the last sample (Sidi Ben Djebbar) is the least effective extract with an inhibition rate not exceeding 60% with antiradicalor activity $1/IC_{50}$ = 2,389 mg/g DM (Table 2).

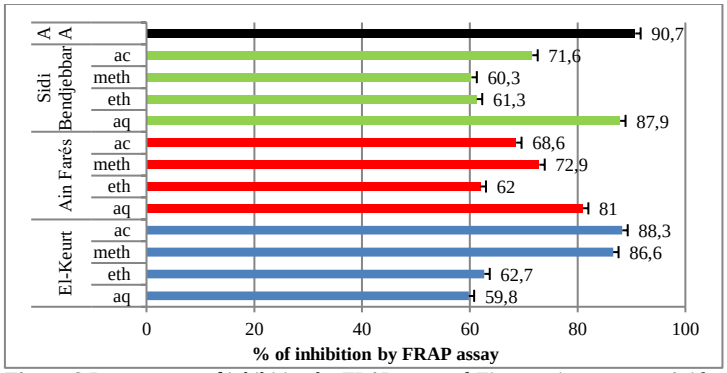

Figure 6 *Pourcentage of inhibition by FRAP essay* of *Ficus carica* extracts. *Acid ascorbic (AA) was used as standart.*
aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.

Li fu et *al*., (2010) studied 56 plants from China including 2 fig species: *Ficus benjamina* and *Ficus hispida* to reveal their antioxidant activity using the iron reduction method which gave IC_{50} varying from 8.26 ± 0.51 µmol Fe(II)/g to 6.26 ± 0.28 µmol Fe(II)/g respectively. The values obtained by Isaac Kingsley Amponsah in 2012 are more effective varying from 186.10 ± 0.012 to 760.00 ± 0.023 µg / ml for the extracts with different solvents. The percentage of inhibition of DPPH (78.65%) by the *Ficus asperifolia* species is higher than that found by the FRAP method (59.27%). These results are similar to our results since the IC_{50} obtained by the DPPH method are the smallest and therefore the antioxidant activity is more important (Ojo and Akintayo, 2014).

Table 2 IC_{50} (mg/g of DM) and $1/IC_{50}$ (mg/g -1 of DM) values obtained in DPPH free radical scavenging assay and FRAP assay.

		DPPH		FRAP	
		IC_{50}	$1/IC_{50}$	IC_{50}	$1/IC_{50}$
Ascorbic Acid	/	**0,0061**	**163,934**	**0,0171**	**58,479**
El-Keurt	Aq	0,158	6,305	1,241	0,805
	Meth	0,101	9,842	1,909	0,523
	Eth	0,078	12,787	1,5	0,666
	Ac	0,189	5,265	1,905	0,524
Ain Farés	Aq	0,293	3,403	1,771	0,564
	Meth	0,207	4,812	1,412	0,708
	Eth	0,215	4,638	1,412	0,708
	Ac	0,241	4,144	1,179	0,847
Sidi Ben Djebbar	Aq	0,403	2,479	2,389	0,418
	Meth	0,187	5,327	1,409	0,709
	Eth	0,406	2,458	1,327	0,753
	Ac	0,235	4,242	1,623	0,615

Ascorbic acid 'AA' was used as reference standard, aq=aqueous ; eth=ethanolic ; meth=methanolic and ac=acetonic.

Correlation: Table 3 shows linear relationship between antioxidant compounds and antioxidant activities; the less is the IC_{50}, the higher is the antioxidant activity. Phenolic compounds appear to contribute significantly positive correlation to the TAC, DPPH and FRAP essay especially total tannins compounds (TTC) that had a strong correlation with DPPH radical assay for two samples (El-Keurt and Ain Farès) also with FRAP assay for the second sample (R^2=0.7855). Previous studies on other samples confirmed our findings (Wahid et *al*., 2010, Debib et *al*., 2013).

Table 3 Correlation between the different activities

		El-Keurt	Sidi Bendjebbar	Ain Farés
TAC	TPC	0,0022	0,3903	0,6429
	TFC	0,9541	0,1965	0,0021
	TTC	0,3404	0,0043	0,1009
FRAP	TPC	0,4274	0,62	0,9328
	TFC	0,3754	0,5924	0,0672
	TTC	0,018	0,7855	0,163
DPPH	TPC	0,0013	0,9692	0,2262
	TFC	0,4481	0,0061	0,0912

TTC	0,9509	0,2294	0,8169

Qualitative and Quantitative Determination of Phenolic Compounds in Fig Extracts by HPLC

The extracts of *Ficus carica* were determined quantitatively by a method. Identification and quantitative determination of the phenolic compounds in the extract were performed by HPLC-UV comparing the retention times and areas with the standard used. This part of study was realised in the Catholic University of Murcia (Spain).

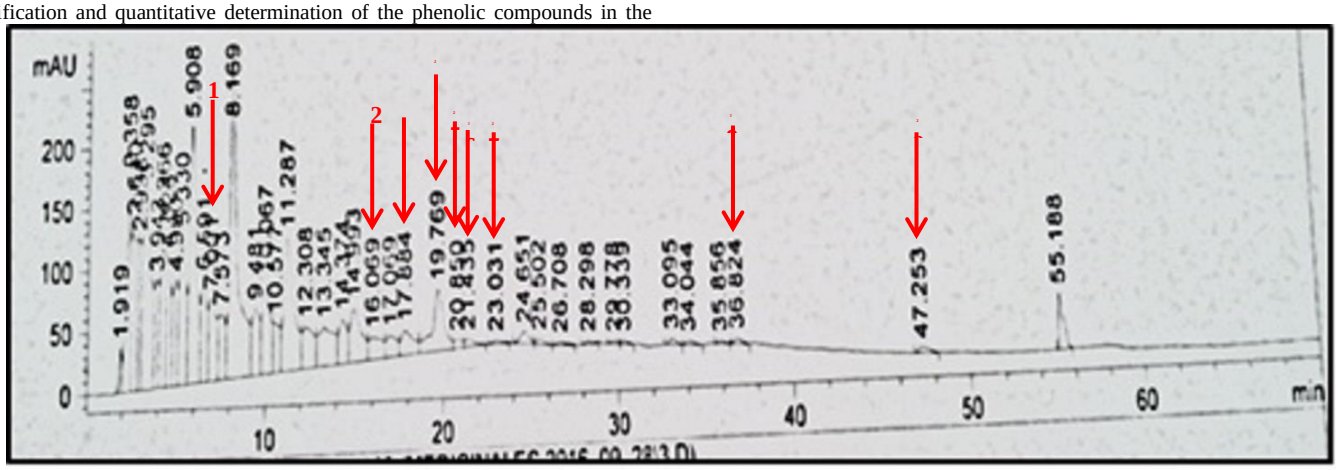

Figure 5 HPLC-DAD phenolic profile of aqueous extract of "El-Keurt" sample. Detection at 280 nm. Peaks:*1,gallic acid; 2, Catechin lidrata /catequin /Epigalocatechin viego;3,Procianidine B2; 4,cafein;5,vanillin ; 6,quercetin ;7,epicatequin ; 8,gencond /Resreratol ; 9, korempferol*.

According to the results obtained in Fig. 5, the phenolic content composition of *Ficus carica* fruits remained incomplete since the main peaks have still not been determined. In addition, the technique we used for HPLC characterization as well as the conditions of analysis was unique and difficult to compare with the values of the literature and previous work.

Antibacterial activity

The antibacterial activity of aqueous and organic extracts shown in Table 04 was studied by determination of the minimum inhibitory concentration (MIC). These results showed that *Citrobacter freundii* was the most sensitive germ with MIC = 1.171 µg/ml but *Listeria innocua* was the most resistant germs with MIC more than 75 µg/ml follow by *Enterococcus feacalis* and *Vibrio cholera* with minimal bactericidal concentration equivalent to 300 µg/ml.

Table 4 antibacterial activity of *Ficus carica* extracts

Strain	G	ZI	MIC	MBC	MBC/ MIC	Extract the most effective
Listeria innocua	06	04	75	300	4	el-keurt ethanolic
Bacillus subtilis	15	11	4.68	150	32.05	el-keurt, sidi bendjbbar ethanolics
Clostridium perfringens	13	15	2.34	75	32.05	bendjebbar ethanolic, el-keurt acetonic
Enterococcus faecalis	11	07	37.75	150	7.94	el-keurt, sidi bendjbbar ethanolics
Staphylococcus aureus	11	10	18.75	150	8.01	methanolic extracts
Vibrio cholera	14	12	37.75	300	7.94	el-keurt acetonic
Pseudomonas aeruginosa	10	08	18.75	300	16.02	el-keurt, sidi bendjbbar ethanolics
Escherichia coli	12	10	2.34	75	32.05	ethanolic, acetonic extracts
Enterobacter sakazakii	13	15	9.37	150	16.02	el-keurt acetonic
Enterobacter cloacae	15	10	2.34	75	32.05	el-keurt acetonic
Proteus mirabilis	09	10	2.34	150	64.1	ethanolic and acetonic of el-keurt
Citrobacter freundii	14	12	1.17	37.5	32.05	el-keurt, sidi bendjbbar ethanolics
Klebsiella oxytoca	12	11	4.68	75	16.02	acetonic extract of sidi Bendjebbar
Serratia odorifera	16	15	2.34	150	64.1	methanolic extracts

G: gentamycin as a positif control in mm**, ZI:** Zone of inhibition by disc diffusion method en mm, **MIC**: minimum inhibitory concentration in µg/ml, **MBC:** minimal bactericidal concentration in µg/ml.

In the work of Jasmine R et *al*. (2014), the ethanol extract of *Ficus carica* showed an antimicrobial activity more than the methanol extract which corresponds to our results because ethanolic extracts especially El-Keurt and Sidi Bendjebbar were the most effective extracts and they can inhibit the majority of germes tested exclusively gram-negative bacteria.

K. pneumonae and *E.coli* revealed a significant resistance to the ethanolic extract of the figs in the study of Rashid and coll. (2014) highlighting the results that were found since these two strains had MBCs of 75 mg / ml and inhibition zones equal to 12 mm for each strain but this does not conform to the work of Kumar et *al*., 2013, where the ethanolic extract of *Ficus palmata* was effective only against *E. coli* without any inhibition zone for *S. aureus* also shown in the table above where the methanolic extract allowed its inhibition.

Truchan et *al*. (2015) worked on different *Ficus* species to evaluate its antibacterial activity against *Pseudomonas aeruginosa* revealing a range of inhibition between 10-15 mm unlike our study where the zone of inhibition did not exceed 10 mm for our extracts as well as that of the standart gentamycin. This is also confirmed by the experience of Ladipo et *al*.in 2011 stating that *P. aeruginosa* and *E.coli* have the highest MIC with 2 mg / ml for each against aqueous extracts of the leaves of *F. exasperata* which does not correspond to our results as showed in table 4.

The minimum inhibitory concentrations of methanolic extracts of *Ficus carica* against *Listeria innocua*, *Enterococcus feacalis* and *Bacillus subtilis* were th same with 3250 µg/ml (Okmen et *al*., 2014) which confused our study since these strains are the most resistant strains especially for the first strain (*Listeria innocua*) where the IMC is 75 µg/ml confirmed also by the aqueous and methanolic extracts of *Ficus sycomorus* latex that did not report any activity against *E.feacalis* just a moderate activity of ethanolic extract (Salem et *al*., 2014).

According to Ravishankar et *al*. (2012), *Ficus bengalensis* revealed significant activity against a group of gram positive and negative bacteria especially *Klebsiella pneumonia* with more than 20 mm of inhibition to be the most sensitive strain. These results remains far from ours since the strain *Klebiella oxytoca* shown some resistance to our extracts which reveal a bacteriostatic activity on this strain giving a ratio MBC/MIC more than 16. Or strains with a CMB / MIC ratio more than 32 this reveals a tolerance of the strain to our extract as *B. subtilis, C. perfringens, E. coli, E. cloocae* and *C. freundii*.

The FIC method that revealed the combination between our *Ficus carica* extracts and gentamycin against the strains studied, the results indicate a synergy and additivity between these extracts and the ATB since the FIC index is varied between 0.5 to 1. The work of Young-Soo and Cha (2010) confirmed this because

they found a synergy and additivity between the extracts of ficus carica and staph aureus resistant to methecillinnot to mention the work of Hosainzadegan et al. (2012) who had the same result studying *S.aureus, E.coli, P. aeruginisa* and *K. pneumoniae* and Jeong and coll. in 2009 working on oral bacteria.

DISCUSSION

In the present study, free radical scavenging potential and total phenolic content of three samples of *Ficus carica* consumed in Mascara were evaluated. Phytochemical investigation of the twelve extracts revealed the presence of chemical constituents that serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Karlovsky, 2008), such as flavonoids, tannins, coumarines, alkaloids and anthocyanins. This diversity has been reported in Adeshina et *al*. (2009), Solomon *et al*. (2006); Oliveira *et al*. (2009). Recent studies have shown that polyphenol levels can be influenced by many environmental factors such as light intensity, mineral nutrition, dryness, and temperature fluctuation (Jaakola et al., 2002, Rabhi et *al*., 2013).

It was found that the best solvent is acetone according to the figures representing the level of polyphenols, flavonoids and tannins, which was approved by previous authors (Al-Farsi and Lee, 2008; Chaalal et *al*., 2012) and the use of solvents for maceration and extraction is still known as an effective means for isolation and selection of bioactive molecules (Engwa et *al*., 2015). Occasionally, Bay et *al*. (2014) revealed that solvent polarity is a premordial point in the extraction of polyphenols and antioxidants. Cell membranes are destroyed by the increase of acetone concentration that enhances the permeability of the solvent into the solid matrix (Zhang et *al*., 2006; Vatai et *al*., 2009). Besides, this solvent favored the extraction of less polar components (Cheok et *al*., 2012) but according to Motlhanka et *al*. (2012), methanol is very good for solvent extraction of polyphenols, tannins, flavones, anthocyanins.
Bucic-Kojic et *al*. (2011) showed that the main contributors of antioxidant capacities include polyphenols but not as much as a major element. For this reason, HPLC-DAD analyses were performed in order to determine the chemical composition of fig fruits in terms of polyphenols content. The data reported in figure 5, shows that El-Keurt fig sample is characterized by the presence of gallic acid, catequin, epigalocatechin, procyanidin, cafein, vanillin, quercetin, epicatequin and korempferol as the two other samples with moderate concentrations in agreements with Slatnar et *al*., 2011 that determined that the presence of this coumpounds can be influenced by sun-dried fruits which may affect the rate of organic acids, sugars, chlorogenic acid, catechin, epicatechin, kaempferol-3-O-glucoside, luteolin-8-C-glucoside, and total phenolic contents. In literature, the drying process of figs is the main cause of the destruction of phenolic compounds which directly influences their antioxidant effect (Apak et *al*., 2007; Nakilcioglu and Hisil, 2013). Accordingly, there may be a close relationship between the intensity of solar radiation and the biosynthesis of polyphenols in the plant explained by some researchers that long exposure to the sun and the amount of precipitation appear to be mostly involved in this natural phenomenon (Rabhi et *al*., 2013). On the other hand, as mentioned previously, the existence of other undetermined compounds with antioxidant activity (Faleh et *al*., 2012), such as those involved in the Maillard reaction (Billaud et *al*., 2005) can not be overlooked. The antioxidant capacity of phenolic compounds results from the high redox potential and the ability to eliminate electron or hydrogen atoms from free radicals, which causes a break in the reaction chains, which then generates oxidative stress (Tsao & Deng, 2004). The statistical analysis confirmed that the tested samples had different antioxidant powers because the highest antioxidant capacity was observed in the El-Keurt sample and the lowest in the Ain Farès sample. this diversity is probably due to the different techniques used for the study of the antioxidant power, difference of extractive technique and maceration by solvent without forgetting the effect of the interfering substances (ascorbic acid, saccharides and / or possibly carotenoids) (Stratil et al., 2007). But for Li Fu et *al*. (2010).These activities reflect an influence by certain factors such as the type of solvent and its polarity, the system used. A reliable antioxidant assessment protocol requires the measurement of several properties because most natural antioxidants are multifunctional. It is therefore essential to make a global determination of the different antioxidants present in the plant to implement the various antioxidant mechanisms (Wong et al., 2006).
According to the available literature, there is no conclusive work confirming the correlation between phenol content and antioxidant capacity (Yu et al., 2002; Ruberto et al., 2007; Konan et al., 2014) but others have found a strong relationship between the two [Pinelo et al., 2005; Konyaloglu, 2005; Makris et al., 2007). It is uncertain which of the phenols and flavonoids exhibit the greatest antioxidant effect but no doubt that quercetin is a flavonoid with high antioxidant and biological properties (Puoci et *al*., 2011), epicatechin and catechin revealed significant biological activity and even preventive activity against cancer (Jankun et *al*., 1997), catechin studied by Graziani et *al*. (2005) was also prevented oxidative damage of human gastric epithelial cells, gallic acid and its glycosides are extremely well absorbed into the human body, compared with other polyphenols (Manach et *al*., 2005) and shows high antioxidant against cancer cells proliferation (Tomas-Barberan et *al*., 2000).

The antimicrobial activity of fig fruits may be due to the presence of several active principals previously mentioned explained by the fact that the ethanolic extract of El-Keurt had a remarkable antimicrobial activity against the majority of strains studied as well as the best antioxidant activity using both TAC and DPPH method also shown in many works that the ethanolic extract gave satisfactory antimicrobial activity (Sharma and Sharma, 2010). Generally, researchers have found that organic extracts (alcoholic) have a more remarkable inhibitory effect than aqueous extracts can be explained by the fact that alcohol is the best solvent for the extraction of active compounds (Jouda et *al*., 2015). Ethanol has always been the most effective solvent because of the high content of phenolic compounds. They attributed this observation to the high volatility of ethanol, which tends to extract more active compound from the sample than water (Ladipo et *al*., 2011).
The high antimicrobial act may be related to the presence of tannins, saponins, alkaloids and flavone aglycones (Jouda MM et al., 2015) and others like rutin, quercetin, luteolin, phenolic acids and phytosterols (Jeong et al.,2009; Rashid et al., 2014)
The overlap in the results of antibacterial activity from one plant extract to another can be attributed to the age of the plant used and therefore freshness of the plant materials studied, environmental factors (temperature, water, lighting), the season and time of harvest and also the drying method used before the extraction process (Jouda et al., 2015) or may be explained by the differences in cell well composition and or inheritance genes on plasmids that can be easily transferred among bacterial strains (Lazrag-Aref et *al*., 2010).
It is possible that these bacteria, both gram positive and gram negative responded well to the plants as they had not been exposed to the plants before, and therefore had not had the opportunity to develop resistance yet as they have to antibiotics over the years. (Jasmine et al., 2014)

CONCLUSION

The analysis of the three algerian *Ficus carica* samples (El-Keurt, Ain Farès and Sidi Bendjebbar) reveals a rich phytochimical profil and a high antioxidant effect that can be reported to the presence of various bioactive compounds (phenolic compounds, flavonoids and tannins). This study highlights traditional medicines for its therapeutic benefits specially the antimicrobial side. Apparently, the potential significance of fig samples studied is therefore as source of antioxidants that could help in reducing the level of oxidative stress and by extension prevents development of chronic diseases.

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