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Evaluation of antimicrobial and antioxidant activities of oily macerates of Algerian dried figs (*Ficus carica* L.)

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Abstract

Phenolic compounds of virgin olive oils and dried figs have been broadly investigated over the past 30 years. Consumption of extra virgin olive oil along with dried fig is a common practice in the Algerian diet, but no data is available regarding their combined biological activities. Recently, we used different Algerian dried fig varieties to obtain virgin olive oily macerates and assess their antioxidant and antimicrobial potential. This article states the estimation of phenolic content of various extra virgin olive oils and their oily macerates prepared from different Algerian varieties of several geographical areas using Folin-Ciocalteu. The process was followed by the evaluation of antioxidant and in vitro antimicrobial potential of the methanolic extracts of oils and macerates against specific resistant human pathogens. The results showed that, the extract of *Chemlali* Bejaïa virgin olive oil (CBOO) variety had notably higher total phenolics (700 ± 56mg of gallic acid/kg) in opposition to *Chemlali* Oran virgin olive oil (COOO)(202.96 ± 26 mg of gallic acid/kg).The extracts of macerates proved to have higher antioxidant and antimicrobial activity against tested Gram-positive and Gram-negative bacteria in comparison with the virgin olive oil extract. Their broad extensive antimicrobial potential may be due to the synergetic effect of polyphenols of both extra virgin olive oils and dried figs. *Candida albicans* showed resistant to pure extra virgin olive oil extracts but didn't to the macerate extracts.

Introduction

In recent decades, the antibiotic resistant strains of clinically significant pathogens have increased rapidly leading to the development of novel bacterial strains that are multi-resistant (Coast *et al*., 1996). The World Health Organization 2014 report on global surveillance of antimicrobial resistance revealed that antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in the community and hospitals (WHO, 2014). Consequently, the need for a rapid means to discovery and development of new antimicrobial compounds is evident. Nutritional approach may prove a likely solution to this problem. Many studies have revealed that above mentioned biocompounds are capable on inhibiting and/or delaying the growth rate of a wide range from bacteria and microfungi (Cowan, 1999). So they might be used as functional food for practicing a preventive or curative effect.

The Mediterranean diet being rich in fruits, vegetables and olive oil has long been correlated with a healthy lifestyle and prolonged life (Trichopoulou and Critselis, 2004). *Ficus carica* L. (fig) belongs to the mulberry Moraceae family which is one of the oldest fruits in the world (Vinson, 1999). Earlier reports related to the nutrient composition of dried figs have indicated that being an important source of minerals and vitamins, it is possess the best nutrient score among the dried fruit (Vinson, 1999). Phytosterols are also reported to be present in fig fruit (433 mg/100 g) (Jeong and Lachance, 2001). The fresh and dried figs also offer relatively higher amounts of crude fiber (5.5%, w/w) and polyphenols (Vinson *et al*., 2005). Some latest works have identified the characterization of three hydroxycinnamic acids (3-O- and 5-O-caffeoylquinic acids and ferulic acid), two flavonoid glycosides (quercetin 3-O-glucoside and quercetin 3-O-rutinoside) and two furanocoumarins (psoralen and bergapten) in figs fruit (Oliveira *et al*., 2009; Debib *et al*., 2016).

The current study aimed to authenticate or reject the traditional Algerian diet hypothesis which

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presuming that olive oily macerates of dried figs can fight infectious diseases. Four macerates were prepared, two from extra virgin olive oil and two from Algerian dried fig varieties. Afterwards, polyphenol fraction was extracted, and phenolic compounds and O-diphenol class of virgin olive oils were estimated. Examination of the extract's antioxidant capacity and antimicrobial activity against Gram positive, Gram negative bacteria and Candida albicans were also performed.

Materials and Methods

Oil and dried figs collection source

Two virgin olive oil samples produced from different areas were used in this study; the Chemlali Oran Olive Oil (COOO), Murdjadjo mark, and Chemlali Bejaïa Olive Oil (CBOO) KHODJA mark, are cultivated in the west, and the east of Algeria. The samples were obtained from ITAF (Technical Institute for Fruit Trees) of Mohammadia (Wilaya of Mascara, Algeria) northwest of Algeria and KHODJA importation-exportation Agrogroupe establishments of Bejaïa north east of Algeria. Algerian Varieties of dried figs samples, Taamriout (green) and Azendjar (black) were procured on October 2013 from KHODJA importation-exportation Agrogroupe establishments and confirmed by ITAF (Technical Institute for Fruit Trees) experts.

Preparation of macerations

50 g of dried fruit were reduced to small 2–6 mm pieces by a cylindrical crusher, then mixed and homogenized with the help of electric blender with 200 ml of extra virgin olive oil and stored at dark in a refrigerated temperature at 4°C for 60 days. After the extraction phase established, the oil was separated from crushed dried fig by filtration using Whatman No.1 filter paper. Four dried figs oily macerates; Chemlali Oran-Taamriout Macerate (COTM), Chemlali Oran-Azendjar Macerate (COAM), Chemlali Bejaïa-Taamriout Macerate (CBTM), Chemlali Bejaïa-Azendjar Macerate (CBAM) were prepared as described above.

Analytical Indices of Olive oil

Free acidity (FA), peroxide value (PV), and UV spectrophotometric indices (K232, K270) were evaluated according to the official methods described in Regulation EC 2568/91(EEC1991) of the Commission of the European Union. All parameters were determined in triplicates for each sample.

Extraction of EVOO and maceration phenolic compounds

The oily and macerates phenolic extracts were prepared by dissolving 50 g of oil or filtrate macerate in 50 ml n-hexane. Polyphenols were extracted from these solutions with three 30 ml portions of CH_3OH/H_2O (80/20 v/v). The mixture was shaken each time for 10min, at 3000 rpm. The separation of oil solution and methanol-water solution was carried out by centrifugation for 15 min, at 6000 rpm (Gutfinger, 1981). The extracts were allowed to dry and then the residues were dissolved in 5 ml methanol (for phenolic compounds quantification) or 2 ml DMSO for antimicrobial analysis (Debib *et al*., 2014).

Quantification of phenolic compounds

The concentration of polyphenols in methanolic extract was estimated with Folin Ciocalteau reagent (Gutfinger, 1981; Karaosmanoglu *et al*., 2010).The preparation of the samples included dilution of 0.5 ml methanolic extract, 1 ml Folin Ciocalteau reagent and, after 3 min, 3.5 ml 10% Na_2CO_3 in a 50ml volumetric flask, with nano-pure water . The absorbance of mixtures was measured after 1h and 15 min, at 765 nm against a blank (reagent without samples) with a UV-VIS spectrophotometr. Values of total phenolic compound were estimated by comparing the absorbance of each sample with a standard response curve generated using gallic acid (0, 12.5, 25, 50, 100 and 200 µg/mL). Results were expressed as milligrams of gallic acid equivalents (GAE) per kg of virgin olive oil or per kg of oily filtrate macerate. For each type of extract the spectrophotometric analysis was repeated three times.

Spectrophotometric Determination of O-Diphenols

A 0.5 mL sample of each phenolic extract was dissolved in 20 mL of methanol/water (1:1, v/v), and 4 mL of the resulting solution was added to 1 mL of 5% solution of sodium molybdatedihydrate in ethanol/water (1:1,v/v) and shaken vigorously. After 15 min, the absorbance at 370 nm was measured against a blank (reagent without samples). Gallic acid used for the calibration curve as standard ($r^2 = 0.996$) (Rotondi *et al.,* 2004). The results were expressed in milligrams of gallic acid per kilogram of oil.

Determination of antioxidant activity using the DPPH method

For the estimation of antioxidant activity, DPPH (2,2 diphenyl 1 picrylhydrazyl) free radical scavenging assay was used following the method described by Mansouri *et al.* (2005). DPPH solution prepared by dissolving (2.4 mg) DPPH in methanol

(100 mL). Next, 50 µL of each extract was separated and mixed with the DPPH solution (1.95 mL) in a test tube. After 30 minutes, the absorbance of these solutions was read at 517 nm. The positive control in this assay ascorbic acid and BHT was used at the same concentrations of extracts, and subjected to the same mentioned procedures for the quantification of antioxidant activity.

The percentage radical scavenging activity (RSA) was calculated using the following formula:

$$\text{DPPH Scavenging Effect (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{x100}$$

Microorganisms source

The microorganisms used as test organisms in the screening included: three Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* subsp. *Spizizenii* (ATCC 6633), five Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853), Escherichia coli (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Salmonella enterica* subsp. *Heindelberg* (ATCC 8326), *Klebsiella pneumoniae* (clinical isolated), and one yeast *Candida albicans* (Clinical isolated). The microorganisms were supplied from the laboratory of microbiology of Algerian Pasteur Institute, except *K. pneumonia* and *C. albicans* were obtained from the laboratory of microbiology of Yessad Khaled hospital, Mascara University, Algeria.

Antimicrobial activity assay

For the estimation of antimicrobial activity, the agar disc diffusion assay was used (Debib *et al*., 2014). The extracts were dissolved in Dimethyl Sulfoxide (DMSO) or distilled water. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Sigma, France) surface inoculate by suspension (200 µL) adjusted by McFarland 0.5 method (10^6 CFU / ml). Sterile filter paper discs of 6 mm diameter were impregnated with 20 µL of the extract solution. The plates were incubated at 37°C for 24 h. Gentamycin (15 µg) and amoxicillin (25 µg) were used as positive controls. Negative controls were performed using paper discs loaded with 20 µL of the solvent used DMSO. The antimicrobial activity was evaluated by measuring the zone of growth inhibition surrounding the discs.

Statistical analysis

Values are given as arithmetic means ± standard error of the mean. Data were statistically analyzed by using one-way analysis of variance (STAVIEW version 5.0, Abacus Concepts, Berkeley, CA) and Student's t-test. Pearson's correlation (r value) was used to determine correlation between antioxidant capacity and polyphenol contents.

Results and Discussion

Chemical parameters and total phenolic content

Chemical parameters considered in this work of the two samples Chemlali Bejaïa (KHODJA mark) and Chemlali Oran (Murdjadjo mark (Table 1), were widely within estimated limits (free acidity ≤ 0.8%, peroxide value ≤20 meq/kg, K270≤0.22 and K232 ≤2.50) of Reg. 2568/91 (EEC 1991a., 1991b), so the oils could be labeled as "extra virgin" according to European Union rules. Results differed significantly between the two samples. The CBOO Chemlali Bejaïa (KHODJA mark) extract variety contained a significantly higher amount of total phenolics (700 ± 56mg of GAE/kg of VOO) versus COOO Chemlali Oran (Murdjadjo mark) 202.96 ± 26 mg of GAE/kg of VOO). The effect of storage on these compounds was clearly observed because the COOO sample with long duration registered the lesser extent.

As regards the amount of O-diphenols in our samples, the sample which represented high value of polyphenols represented a higher value of O-Diphenols too. The ratio between O-diphenols and total polyphenols was determined for all samples. A small difference was observed between values but it was not significant.

Algeria, considers one of the main producers of olive oil in the world (9th), It has a wide range of varieties. Recently, phenolic compounds present in olive oil have received much attention due to their beneficial functional and nutritional effects (Laincer *et al*., 2014). Our results confirmed that the polyphenols concentration of extra virgin olive oils may differ, depending on environmental factors and the storage period. Generally polyphenols values in The CBOO Chemlali Bejaïa (KHODJA mark) extract variety are higher than those reported by Karaosmanoglu *et al.* (2010) in Turkish extra virgin olive oils (125.29 - 353.36mg of GAE/kg of oil) and those reported by Laincer *et al*. (2014) in Algerian virgin olive oil (115 – 420.95 mg of GAE/kg of oil). Other studies reported that the polyphenol levels vary, depending on the degree of ripeness of the olives and the extraction procedures followed (Montedoro and Servili, 1992; Di Giovacchino *et al.*, 1994). In accordance with the literature data, the concentration of both the total phenolic and the O-diphenolic fractions extracted from different oil samples indicated a high variability in the phenolic content of the virgin olive oils available on the market. Previous data indicated that

Table 1. Total phenolic content and chemical parameters determined in virgin olive oil (VOO) samples.

Parameter	COOO	CBOO
Date of extraction	Jan18, 2010	Dec 13, 2011
Free acidity (%)	0.69 ± 0.41	0.22 ± 0.021
POV (mequiv of O_2/kg)	11.39 ± 0.04*	9.03 ± 1.21*
K_{232}	1. 33 ± 0.013*	0.38 ± 0.04*
K_{270}	0.08 ± 0.001*	0.12 ± 0.015*
Total phenolic compounds (mg of gallic acid/kg)	202.96 ± 26*	700 ± 56*
O-Diphenols (mg of gallic acid/kg)	27.396 ± 0.007	62.67 ± 0.15
O-DP/TP	0.13	0.08

* Significantly different ($p<0,05$).
CBOO; *Chemlali* Bejaïa (KHODJA mark),
COOO; *Chemlali* Oran (Murdjadjo mark),

olive oil phenols can commonly be divided into three categories: simple phenols, secoiridoids, and lignans, all of which inhibit auto-oxidation. Major phenols include hydroxytyrosol, tyrosol, oleuropein (Perona *et al*., 2006; Laincer *et al*., 2014) and ligstroside (Karaosmanoglu *et al*., 2010; Laincer *et al*., 2014). Hydroxytyrosol and tyrosol are simple phenols whereas oleuropein is a secoiridoid.

Antimicrobial activity

Antimicrobial activity results are summarized in Table 2, globally we found that the virgin olive oil extracts and oily dried figs macerates extract exhibited inhibition zones of about 8-16 mm against certain bacteria, indicating a wide spectrum activity against both gram positive and gram negative bacteria. However, the positive controls were more potent in inhibiting the microbes, while negative control which was DMSO did not show any inhibition zones.

Concerning the phenolic extracts of virgin olive oil, CBOO *Chemlali* Bejaïa virgin olive oil extract exhibited strong activities against *P. aeruginosa* (12 ± 1.03 mm), *S. enterica* (12 ± 0.31 mm), *E. faecalis* (12 ± 0.02 mm) and *S. aureus* (14 ± 1.02 mm) compared with the COOO *Chemlali* Oran oily extract. For the other microbes (*E. cloacae* and *C. albicans*) tested, we registered no effect (Table 2).

The oily dried figs macerates extracts, possessed significantly higher antimicrobial activity as compared with the oily extracts, especially CBAM and CBTM macerates prepared by *Chemlali* Bejaïa virgin olive oil with the highest content of polyphenols. On the other hand COTM and COAM macerates prepared by *Chemlali* Oran olive oil, except slight effects against *E. coli* and *B. subtilis*, did not show any inhibition against the others microbes.

Antimicrobial activity results of this investigation validate the use of olive oily macerates of dried figs by Algerian people to fight infectious diseases. The findings of this study are in agreement with the studies of Laincer *et al*. (2014), who reported large inhibition zones against Gram positive and Gram negative bacteria. On another hand it has been reported that antibacterial activity of olive oil's phenolic compounds is due to the presence of the ortho-diphenolic system (catechol) (Bisignano *et al*., 1999). Therefore, the position and number of hydroxyl groups on the phenol group are thought to be related to their rela¬tive toxicity to microorganisms, with evidence that increasing hydroxylation results in an increase in anti-microbial activity. Oleuropein was significantly less toxic to bacterial cells than hydroxytyrosol; one can speculate that the glycosidic group of oleuro-pein might render the unable to penetrate cell mem¬branes or to reach the target site (Laincer *et al*., 2014).

The oily dried figs macerates extracts, possessed significantly higher antimicrobial activity as compared with the oily extracts. Debib *et al.* (2014) results showed that dried fig extract from Taamriout and Azendjar varieties were rich in tannins, flavonoids, saponins and alkaloids. For that reason, the results presented here are promising, and evidenced that the dried figs bioactive molecules were extracted and were present in the oily macerates extracts. Our results are in agreement of previous reports which showed that coumarins largely present in fig are effective against Gram positive and Gram negative bacteria (Cottiglia *et al*., 2001; Rosselli *et al*., 2007; Marwat *et al*., 2009). Resveratrol, psoralen and the bergapten showed antibacterial activity (Zao *et al*., 2005).

Table 2. Antimicrobial activity of different phenolic extracts of virgin olive oil, dried fig oily macerates and standards antibiotic.

Exract/atb bacteria	CBOO	COOO	COTM	COAM	CBTM	CBAM	AMO	GEN	DMSO
E. coli	8± 0,03	-	8± 1.84	11± 0.01	12± 2.07	14± 1.03	25	28	-
E. cloacae	-	-	-	-	10± 0.67	14± 1.03	00	-	-
P. aeruginosa	12 ± 1.03	8 ± 0.4	-	-	8± 1.32	8± 1.03	-	24	-
K. pneumoniae	10 ± 0.09	12 ± 0.61	-	-	14± 0.01	14± 0.44	15	23	-
S. enterica	12 ± 0.31	-	-	-	-	-	18	25	-
E. faecalis	12 ± 0.02	-	12	-	14± 1.08	16± 0.22	22	23	-
S. aureus	14 ± 1.02	8 ± 0.25	-	-	8± 0.53	10± 0.08	-	26	-
B. subtilis	10 ± 0.09	8 ± 1.12	10± 1.03	10	12± 1.43	12	24	20	-
C. albicans	-	-	11± 2.03	12± 0.18	12± 0.73	14± 1.63	ND	ND	-

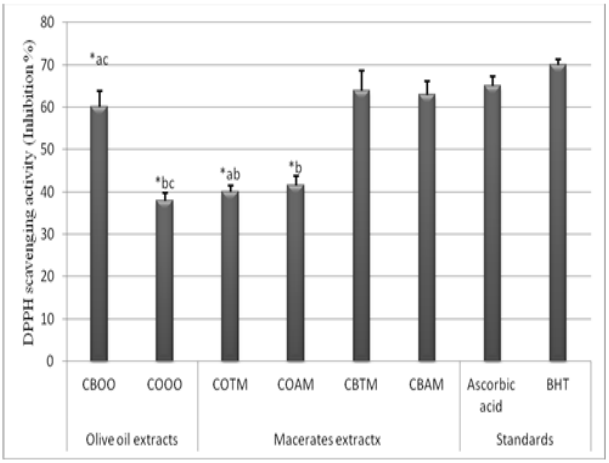
- : No inhibitory effects, CBOO; *Chemlali* Bejaïa olive oil, COOO; *Chemlali* Oran olive oil, COTM; *Chemlali* Oran-Taamriout Macerate, COAM; *Chemlali* Oran-Azendjar Macerate, CBTM; *Chemlali* Bejaïa-Taamriout Macerate, CBAM; *Chemlali* Bejaïa-Azendjar Macerate

Antioxidant activity

The antioxidant activity of study samples is illustrates in Figure 1. The antioxidant activity of CBOO, *Chemlali* Bejaïa olive oil extract was significantly ($p<0.05$) higher than that of COOO (*Chemlali* Oran olive oil) extract. Mixing of dried figs with the two samples significantly ($p<0.05$) increased the antioxidant activity of oily macerates.

The correlation coefficient (r) among the free radical (DPPH) scavenging activity (%), the total phenolics and total O-Diphenols content in CBOO and COOO olive oil extracts was determined. The (DPPH) scavenging activity and the total O-Diphenols content displayed a better correlation in the two sample ($r^2= 0.77$, $p<0.01$, $r^2= 0.64$, $p<0.05$) respectively.

The radical-scavenging activities of our oily dried figs macerates extracts are significantly higher as compared with the radical-scavenging activities oily extracts. This data might depend on the composition and pro-file of phenolic compounds. Where it can be observed that the maceration favored the extraction of active molecules and enhanced the antimicrobial and antioxidant effects, that conducts to hypothesize that a synergistic effect between virgin olive oil phenolic compounds and dried figs phenolic compounds improves antioxidant and antimicrobial activity of figs olive oily macerates.



*a significantly different vs ascorbic acid
*b significantly different vs BHT
*c significantly different vs their macerate

Figure 1. Free radical scavenging activity of different phenolic extracts of virgin olive oil, dried fig oily macerates and standards.

Conclusion

Conclusively, our study suggest that the use of dried figs olive oily macerates as an alternative treatment of infections might be beneficial, in spite of the fact have not been completely explored. Furthermore, it is necessary to isolate the compound and the mechanism that responsible for this antioxidant and antimicrobial activities. It will be the next work of our group.

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