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# Olive oils from Algeria: Phenolic compounds, antioxidant and antibacterial activities

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**SUMMARY:** The phenolic compositions, antioxidant and antimicrobial activities against six bacteria of phenolic extracts of olive oil varieties from eleven Algerian varieties were investigated. The antioxidant activity was assessed by determining the scavenging effect on the DPPH and $ABTS^{\cdot+}$ radicals. The antimicrobial activity was measured as a zone of inhibition and minimum inhibitory concentration (MIC) on human harmful and foodborne pathogens. The results show that total phenols was significantly ($p< 0.05$) correlated with DPPH ($r = 0.72$) and $ABTS^{\cdot+}$ radicals ($r = 0.76$). Among the bacteria tested, *S. aureus* and to a lesser extent *B. subtilis* showed the highest sensitivity; the MIC varied from 0.6 to 1.6 $mg{\cdot}mL^{-1}$ and 1.2 to 1.8 $mg{\cdot}mL^{-1}$, respectively. The results reveal that Algerian olive oils may constitute a good source of antioxidant and antimicrobial agents.

***KEYWORDS:*** *Antibacterial activity; Antioxidant activity; Olive oil; Phenols*

**RESUMEN:** ***Aceites de oliva de Argelia: Componentes fenólicos, actividades antioxidante y antibacteriana.*** Se ha estudiado la composición fenólica y las actividades antioxidante y antimicrobiana, contra seis bacterias, de extractos de aceites de oliva de once variedades argelinas. La actividad antioxidante se evaluó mediante la determinación del efecto captador de radicales de DPPH y $ABTS^{\cdot+}$. La actividad antimicrobiana se midió como zona de inhibición y como concentración inhibitoria mínima (MIC) sobre bacterias perjudiciales humanas y agentes patógenos transmitidos por los alimentos. Los resultados mostraron que los fenoles totales está significativamente ($p< 0,05$) correlacionados con DPPH ($r = 0,72$) y los radicales $ABTS^{\cdot}+$ ($r= 0,76$). Entre las bacterias ensayadas, *S. aureus* y, en menor grado *B. subtilis* mostraron la mayor sensibilidad; el MIC varió de 0,6 a 1,6 $mg{\cdot}mL^{-1}$ y 1,2 a 1,8 $mg{\cdot}mL^{-1}$ respectivamente. Los resultados muestran que los aceites de oliva argelinos pueden constituir una buena fuente de antioxidantes y agentes antimicrobianos.

***PALABRAS CLAVE:*** *Aceite de oliva; Actividad antibacteriana; Actividad antioxidante; Fenoles*

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## 1. INTRODUCTION

The Mediterranean diet is associated with lower incidences of atherosclerosis, cardiovascular disease, neurodegenerative diseases and certain types of cancer. The apparent health benefits have been partially ascribed to the dietary consumption of virgin olive oil by Mediterranean populations (Cicercale *et al*., 2010). The beneficial effects of virgin olive oil were attributed to its high monounsaturated fatty acids particularly oleic acid and minor compounds such as phenolic compounds, tocopherols and carotenoids (Visioli and Galli, 1998).

Recently, phenolic compounds present in olive oil have received much attention due to their beneficial functional and nutritional effects including antioxidant and antimicrobial activities. In addition to extending the shelf life of foods by inhibiting lipid peroxydation, phenolic compounds scavenge free radicals and can consequently protect the human body **(**Cicerale *et al*., 2009). Numerous studies have demonstrated the antioxidant activity of olive oil polyphenols (Visioli *et al*., 2002, Servili *et al*., 2009).

Moreover, several studies have shown that the phenolic compounds in olive oil have a high antimicrobial activity against a broad spectrum of foodborne pathogens (Medina *et al*., 2006; Karaosmanoglu *et al*., 2010). Oleuropein (Bisignano *et al*., 1999; Furneri *et al*., 2004), hydroxytyrosol (Bisignano *et al*., 1999) which inhibits or delays the growth rate of a range of bacteria. Romero *et al.* (2007) reported that the dialdehydic form of decarboxymethyl oleuropein aglycon and the dialdehydic form of decarboxymethyl ligstroside aids in inhibiting the growth of *Helicobacter pylori*. Recently, Bubonja-Songe *et al.* (2011) indicated that olive oil polyphenols can be used as an alternative for the prevention of food spoilage and *monocytogenes* contaminated with *Listeria*. The increasing resistance to antibiotics represents the main factor justifying the need to find out and/or develop new antimicrobial agents. Although strategies have been proposed in an attempt to control the pathogenic bacteria spread, the search for new methods to treat infections stimulates the investigation for natural compounds.

Algeria, one of the main producers of olive oil in the world (9th), has a wide range of varieties. Several studies on Algerian olive oil characterization have been carried out. However, there is no report about antioxidant and antimicrobial activities of phenolic compounds in the olive oil produced in Algeria. The present work was undertaken (i) to identify and quantify the phenolic compounds from various olive oils from Algerian varieties using a reverse-phase high-performance liquid chromatography (RP-HPLC) system and (ii) to evaluate the antioxidant and antimicrobial properties of olive oils obtained from different varieties of olives grown in the Bejaia region (Algeria).

## 2. MATERIALS AND METHODS

### 2.1. Fruit harvest

The extra virgin olive oils used in this work came from eleven different Algerian varieties of olives: *Aghenfas, Akerma, Blanquette de Guelma, Bouchouk Soummam, Bouricha, Chemlal, Ferkani, Limli, Neb Djemel, Tabelout and Chemlal Tazmalt*.

Fruits were manually harvested from an orchard located in Takerietz (Bejaia), during the 2008 and 2009 seasons, with the exception of the *Chemlal Tazmalt* variety which was harvested in the Tazmalt region (Bejaia) in east-central Algeria. Collection, transportation and processing of the olive samples were carried out rapidly and with care. Therefore, only fresh drupes without damage were selected.

### 2.2. Olive oil extraction

Virgin olive oil samples were obtained using a laboratory oil mill (Levi-Deleo-Lerogsame), consisting of three basic elements: a hammer crusher, thermo-beater (mixer) and a pulp centrifuge. The olive fruits were milled in the hammer crusher, and then the olive paste was kneaded for 30 min with the addition of warm water (50 mL of water was added to 920 g of paste). After the vertical centrifugation, the oil was collected and left to stand. The oil samples were stored in amber glass bottles at 4 °C in the dark without headspace until analysis.

### 2.3. Quality indices

Acidity and peroxide values were determined following the analytical methods described by the International Union of Pure and Applied Chemistry (IUPAC, 1979). $K_{232}$ and $K_{270}$ were determined according to the analytical methods described by the International Olive Oil Council (IOOC, 1996).

### 2.4. Liquid–liquid extraction of phenolic compounds

A Liquid–liquid extraction system was carried out in the same extraction conditions, reported by Tsimidou *et al.* (1992) with some modifications. Briefly, 50 g of oil were dissolved in 50 mL of hexane, the solution was extracted successively three times with 30 mL of an MeOH/water (80:20, v/v) solution. The extracts were combined and washed twice with 50 mL of hexane to remove the residual oil. The hexane phase was discarded, and the methanolic solutions were concentrated and dried using rotary evaporator under vacuum at 40 °C. Finally, the residue was re-dissolved in a solution of MeOH/water (80:20, v/v).

### 2.5. Determination of total phenols

The total phenolic content of the extract was determined by the Folin-Ciocalteu spectrophotometric method at 765 nm, using gallic acid as calibration curve (Favati *et al*., 1994).

### 2.6. Determination of *O*-diphenols

According to Bendini *et al*. (2003), 0.5 mL of phenolic extract obtained from olive oil were dissolved in 5 mL of MeOH-water (1:1, v/v); a mixture of 4 mL of the solution and 1 mL of a 5% solution of sodium molybdate dihydrate in MeOH-water (1:1, v/v) was shaken vigorously. After 15 min, the absorbance at 370 nm was measured using caffeic acid for the calibration curve with a glass cuvette.

### 2.7. Chromatographic determination of phenolic compounds

A solution of internal standard (1 mL of 0.015 $mg \cdot mL^{-1}$ of syringic acid in water/MeOH (20:80 v/v) was added to a sample of virgin olive oil (2 g). The mixture was shaken (30 s) and 5 mL of extraction solution water and MeOH (20:80 v/v) were added. The obtained mixture was shaken for 1 min, extracted for 15 min in an ultrasonic bath and then centrifuged at 5000 r.p.m. (2500 g) for 25 min at T= 20 °C (NGD., 2010). The upper phase was filtered with a 0.45 µm PVDF syringe filter. 20 µL of the filtered solution were analyzed by HPLC with a UV detector at 280 nm. The HPLC system used consisted of a C18 Spherisorb ODS-2 reverse column (5 µm, 250 mm, 4.6 mm). Elution was performed at a flow rate of 1 $mL \cdot min^{-1}$ following a gradient composed of a mixture of water and orthophosphoric acid (99.8:0.2 v/v) (solvent A), MeOH (solvent B) and acetonitrile (solvent C): from 96% (A) – 2% (B) – 2% (C) to 0% (A) – 50% (B) – 50% (C) in 60 min. The last gradient was kept for 10 min. The successive gradient was: from 0% (A) – 50% (B) – 50% (C) to 96% (A) – 2% (B) – 2% (C) in 2 min and then kept for 10 min.

The identification of phenolic compounds was performed by HPLC-MS. The main phenolic compounds were identified by comparison with relative retention times and UV spectra of pure standards.

### 2.8. Determination of antioxidant activity

#### *2.8.1. DPPH radical- Scavenging assay*

The capacity of methanolic extracts of olive oil to reduce the DPPH (2,2-diphenyl-picrylhydrazyl) radical was assessed using the method of Keceli and Gordon (2001). 0.1 mL of a methanolic extract were added to 2.9 mL of a solution of DPPH ($6.10^{-5}$ M) in methanol for 30 min and the absorbance was recorded at 515 nm. The scavenging activity of the extracts was evaluated according to the formula:

$$\text{percent of scavenging} = [A_0\text{-}(A_1 – A_S)]/\ A0]100$$

where $A_0$ is the absorbance of DPPH alone, $A_1$ is the absorbance of DPPH + extract and $A_S$ is the absorbance of the extract alone.

#### *2.8.2. Scavenging activity against the $ABTS.^+$ radical cation*

The total antioxidant activity of extracts was measured by the $ABTS^{\cdot+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay of Re *et al.* (1999) with minor modifications. $ABTS^{\cdot+}$ was generated by the oxidation of ABTS with potassium persulfate. Prior to assay, the $ABTS^{\cdot+}$ stock solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. Then 990 µL of a diluted $ABTS^{\cdot+}$ solution was mixed with 10 µL of the test sample, and the absorbance was measured at 734 nm after 30 min. The inhibition percentage of absorbance was calculated using the formula specified in the DPPH method. The antioxidant capacity of test compounds was expressed as $IC_{50}$, the concentration necessary for 50% reduction of $ABTS^{\cdot+}$

### 2.9. Determination of antibacterial activity

#### *2.9.1. Bacterial strains and culture conditions*

The bacterial strains used as test organisms were *Escherichia coli* Nalidixic Acid Resistant NAR**,** *Klebsiella pneumoniae* E47, *Listeria innocua* CLIP 74915, *Pseudomonas aeruginosa*; ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. The bacteria were cultured at 37 ºC in a nutrient broth and agar medium. Before experimental use, the cultures from solid medium were sub-cultivated in a liquid medium, incubated for 18 h and used as the source of inoculums for each experiment.

#### *2.9.2. Antibacterial Activity Assay*

The agar diffusion method (Kappel *et al*., 2008) was used for the antibacterial assay. Suspensions of the microorganisms were prepared to contain approximately $10^8$ CFU/mL then 0.1 mL of the test organisms were inoculated with a sterile swab on the surface of the Mueller–Hinton agar. Sterile paper disks (6 mm in diameter) (REF-NO: 321 261, Antibiotica-testblattchen *D 3354.* Dassel W-Germany) were impregnated with 20 µL of the extract solutions in order to obtain final concentrations of 1, 0.5, 0.25 and 0.12 mg, respectively, of extract in the disks. Negative controls were prepared

using the same solvent employed to dissolve the samples (Methanol/water (80:20, v/v)). Standard reference antibiotics, gentamycin (15 µg), chloramphenicol (30 µg) and ofloxacin (5 µg) were used as positive controls for the tested bacteria. After incubation for 18–24 hours at 37 °C the diameters of the zone of inhibition (without diameter of the disc) were measured and expressed in mm. The presence of a zone of inhibition indicates the activity of tested extracts against bacteria.

Minimum inhibitory concentrations (MICs) were also investigated for the bacterial strains which were determined as sensitive to the compounds in the disc diffusion assay; the minimum inhibitory concentration (MIC) for each extract tested was determined using the agar dilution technique (Tunçel and Nergiz, 1993). Appropriate amounts of each extract were aseptically added to the super-cooled sterile Mueller–Hinton to give final concentrations of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.4, 1.6, 1.8 and 2 mg·mL$^{-1}$. Approximately $10^8$ CFU mL$^{-1}$ of each culture were inoculated as a spot onto the Mueller–Hinton containing the desired extract using a 1 µL calibrated loop and the plates were incubated at 37 °C for 48 h. The minimum concentration without bacterial growth was determined to be the MIC.

### 2.10. Statistical analysis

The data reported were subjected to analysis of variance using the Statistica 5.5 package (StatSoft 97 edition). Where statistical differences were noted, differences among packages were determined using the ANOVA/MANOVA following the Newman-Keuls test. Significance was defined at $p<0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Quality Indexes

For the majority of the oils analyzed, the mean values of the quality parameter (acidity, peroxide index, and specific ultraviolet absorbance) fell within the ranges allowed by the IOC norms (2003) for the extra-virgin olive oil category (free acidity ≤ 0.8%, peroxide value ≤20 meq·kg–$^{1}$, $K_{270}$≤0.22 and $K_{232}$≤2.50).

The peroxide value and acidity of the studied oil were in the ranges of 3.25–8.75 meq·kg–$^{1}$ and 0.05–0.23 (% oleic acid), respectively. $K_{270}$ was between 0.107 and 0.206 and $K_{232}$ was in the range of 1.155 to 2.461.

### 3.2. Total phenols and *O*-diphenols

As shown in Table 1, the amount of total phenols and *O*-diphenols in the analyzed oils varies significantly ($p$≤0.05) among the different cultivars. The results show that the amounts of total phenols and *O*-diphenols are highly variety-dependent. The average folin-ciocalteun content of total phenols in the samples analyzed was 218.66 mg·kg$^{-1}$, although a wide range of concentrations was observed, from 115 mg/kg (*Chemlal Tazmalt* variety) up to 420.95 mg·kg$^{-1}$ (*Neb Djemel* variety). The extract from the *Neb Djemel* variety had the highest contents of phenols and *O*-diphenols (420.95 and 29.67 mg·kg$^{-1}$, respectively), whereas, the extracts from the *Chemlal Tazmalt* variety (115.73 and 7.54 mg·kg$^{-1}$, respectively) and *Limli* variety (123.43 and 5.68 mg·kg$^{-1}$, respectively) had the lowest (Table 1). Our results are higher than those found by Taniglan *et al.* (2007) for several Turkish olive oil varieties. A significant positive correlation between total phenol and *O*-diphenol contents was observed ($r=0.88$, $p<0.05$). These results are in accordance with the correlation coefficient reported by Cerretani et al. (2006).

### 3.3. HPLC profiles of phenols

According to the classification of Montedoro *et al.* (1992), the total phenol contents determined by HPLC (Table 2) can be classified as high for *Neb Djemel, Blanquette de Guelma and Bouricha*, mid-range for *Akerma, Chemlal, Chemlal de Tazmalt, Ferkani, Limli* and *Tabelout* and low for *Aghenfas* and *Bouchouk Soummam*.

The identified phenolic compounds are mainly secoiridoid derivatives, phenolic alcohols, flavonoids, and lignans. The main simple phenols found in the analyzed olive oils were hydroxytyrosol and tyrosol. The concentration of tyrosol varied from 2 to 28 mg·kg$^{-1}$ in all analyzed samples, which was higher than that of hydroxytyrosol (0.5–5 mg·kg$^{-1}$). These results agree with previous data found in

Table 1. Total phenols and O-diphenols (mean±SD, n=3) in different Algerian varieties of virgin olive oil

| Varieties | Total phenols (mg·kg–1) | O-diphenols (mg·kg–1) |
|---|---|---|
| Aghenfas | 161.78±6.25$^{c}$ | 13.15±0.00$^{d}$ |
| Akerma | 130.93±7.68$^{b}$ | 10.07±0.00$^{c}$ |
| Blanquette de Guelma | 365.25±8.41$^{f}$ | 23.37±0.59$^{g}$ |
| Bouchouk Soummam | 152.17 ± 7.24$^{c}$ | 10.92±0.00$^{c}$ |
| Bouricha | 369.79±6.88$^{f}$ | 15.65±0.86$^{e}$ |
| Chemlal | 234.38±6.43$^{d}$ | 15.49±0.18$^{e}$ |
| Chemlal Tazmalt | 115.73±4.36$^{a}$ | 7.54±0.00$^{b}$ |
| Ferkani | 243.23±2.87$^{e}$ | 19.30±0.00$^{f}$ |
| Limli | 123.43±0.79$^{ab}$ | 5.68±0.00$^{a}$ |
| Neb Djemel | 420.95±2.03$^{g}$ | 29.67±0.73$^{h}$ |
| Tabelout | 239.79±1.69$^{e}$ | 19.36±0.97$^{f}$ |

$^{a–h}$ Means the column followed by different letters are significantly different ($P<0.05$).

Tunisian varieties (Haddada *et al*., 2008) and Spanish and Greek varieties grown in northern Tunisia (Allalout *et al*., 2009). Tyrosol was the main phenolic component present in the *Blanquette de Guelma* variety, with 28 mg·kg$^{-1}$ (Table 2).

The most abundant secoiridoids of the olive oil analyzed were secoiridoid derivatives of oleuropein and ligstroside; these results agree with previous data found for Spanish varieties (Brenes *et al*., 2000; Oliveras-Lopez *et al*., 2007). The content of oleuropein and ligstroside derivatives ranged from 50 to 26 mg·kg$^{-1}$ in *Aghenfas* oils to 276 and 173 mg·kg$^{-1}$ in *Neb Djemel* oils, respectively.

The amount of derivatives of oleuropein in all the analyzed samples was higher than that of the derivatives of ligstroside except for the *Akerma, Limli* and *Tabelout* varieties which showed higher contents of the derivatives of ligstroside (44, 62 and 124 mg·kg$^{-1}$, respectively). Arslan, (2012) noted that oleuropein was the most abundant phenolic in the olive oil of Turkish varieties.

Flavonoids are present in considerable amounts with concentrations varying from 10 mg·kg$^{-1}$ for the *Limli* variety to 44 mg·kg$^{-1}$ for the *Aghenfas* variety. These results were higher than those reported by Ilyasoglu *et al.* (2010). The main flavonoid compounds quantified in our study were luteolin and apigenin. Considerable quantitative differences in their concentrations among the different varieties were noted. The luteolin amount ranged from 6 mg·kg$^{-1}$ for the *Tabelout* variety to 38 mg·kg$^{-1}$ for the *Akerma* variety. Apigenin was present at a high level in the *Chemlal Tazmalt* variety (14 mg·kg$^{-1}$) compared to the others.

Lignans were also present in significant amounts in all the analyzed samples at concentrations ranging from 12 mg·kg$^{-1}$ in the *Bouchouk Soummam* variety to 51 mg kg$^{-1}$ in the *Bouricha* variety. Although the variety *Neb Djemel* showed a high level of total polyphenols (537 mg kg$^{-1}$), it contains only 21 mg·kg$^{-1}$ of lignans, on the other hand, the *Chemlal Tazmalt* variety, which is less rich in total polyphenols (257 mg·kg$^{-1}$) showed a high content of lignans (41 mg·kg$^{-1}$).

Lignans are the main component of the phenolic fraction of the olive seed and are practically absent in the pulp, leaves, and limbs, therefore their presence in the oil must be due to the breaking of the stones when the olives are crushed (Owen *et al*., 2000).

The presence of elenolic acid in olive oil in its free form is due to the hydrolysis of oleuropein, ligstroside, and related glucosidic compounds. The *Neb Djemel* variety was distinguished by a high concentration of elenolic acid (68.4 mg kg$^{-1}$), the lowest value was noted for the *Blanquette de Guelma* variety (13.3 mg·kg$^{-1}$). This compound was found at a low concentration compared to the varieties cultivated in Chile and Spain (García-González *et al*., 2010) which did exceed 115 mg·kg$^{-1}$.

Recently, an interest in the oxidized form of virgin olive oil phenols has significantly increased, especially in relation to determination of freshness/ageing status (Rovellini and Cortesi, 2002). The ratio between oxidized and natural forms is the key for a good quality judgment (Rovellini, 2008). A high amount of oxidized phenols was noted in the *Chemlal Tazmalt* variety (67 mg·kg$^{-1}$).

Table 2. Phenolic compounds composition obtained from Algerian virgin olive oil (mg·kg$^{-1}$), by HPLC analysis

| | Aghenfas | Akerma | Blanquette de Guelma | Bouchouk Soummam | Bouricha | Chemlal | Chemlal Tazmalt | Ferkani | Limli | Neb Djemel | Tabelout |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oleuropein derivatives | 50 | 36 | 203 | 82 | 235 | 133 | 61 | 119 | 60 | 276 | 120 |
| Ligstroside derivatives | 26 | 44 | 115 | 32 | 150 | 101 | 50 | 91 | 62 | 173 | 124 |
| Hydroxytyrosol | 0.5 | 0.9 | 5 | 2 | 5 | 3 | 2 | 1 | 3 | 4 | 4 |
| Tyrosol | 3 | 4 | 28 | 2 | 26 | 3 | 3 | 4 | 26 | 5 | 11 |
| Total Flavonoids | 44 | 49 | 18 | 41 | 25 | 23 | 34 | 14 | 10 | 33 | 13 |
| Apigenin | 12 | 11 | 6 | 12 | 7 | 8 | 14 | 4 | 3 | 13 | 7 |
| Luteolin | 32 | 38 | 12 | 29 | 18 | 15 | 20 | 10 | 7 | 20 | 6 |
| Lignans | 16 | 38 | 33 | 12 | 51 | 38 | 41 | 42 | 25 | 21 | 29 |
| Natural Elenolic acid | 33.6 | 27.2 | 13.3 | 23.2 | 58.3 | 24.2 | 29.9 | 42.9 | 34.7 | 68.4 | 27.7 |
| Oxidized elenolic acid | 0.20 | 0.12 | 0.11 | 0.02 | 0.09 | 0.05 | 00 | 0.02 | 0.07 | 0.02 | 0.20 |
| Phenols oxidized | 14 | 15 | 51 | 18 | 55 | 54 | 67 | 13 | 42 | 19 | 22 |
| Total phenols (HPLC) | 169 | 207 | 430 | 197 | 522 | 354 | 257 | 287 | 208 | 537 | 316 |

The concentration of oxidized elenolic was very low in all varieties and was completely absent in the *Chemlal Tazmalt* variety, the highest values were noted for the *Aghenfas*, *Tabelout* varieties.

### 3.4. Antioxidant activity

#### *3.4.1. DPPH assay*

The results of DPPH radical-scavenging activities (Table 3) showed significant differences ($p < 0.05$) among the varieties. The *Blanquette de Guelma* variety exhibited the best DPPH scavenging activity (72.20%). The extracts of *Limli*, *Akerma* and *Chemlal Tazmalt* oils recorded the lowest percentages (39.96, 38.20 and 36.57% respectively). These values were similar to those reported by Nakbi *et al.* (2010) for Tunisian cultivars (78.56 and 37.23% for *Chetoui and Chemlali* varieties, respectively).

A significant correlation ($p<0.05$) is noted between DPPH radical-scavenging activity and phenolic compound content ($r=0.72$, $p<0.05$) and *O*-diphenols ($r=0.67$, $p<0.05$). These coefficients are in agreement with the report by Samaniego-Sánchez *et al.* (2007) with the coefficient $r=0.79$ ($p<0.05$) between total polyphenol and DPPH radical-scavenging activity.

The different radical-scavenging activities of our extracts might depend on the composition and profile of phenolic compounds among the varieties, particularly *O*-diphenols. The oleuropein derivatives were the main compounds found in eight varieties. Their antioxidant properties can be related to hydrogen donation and their ability to improve radical stability by forming an intra-molecular hydrogen bond between the free hydrogen of their phenoxyl radicals. The presence of a second hydroxyl group at the ortho-position significantly enhances their ability to act as an antioxidant as measured by the DPPH radical test.

On the other hand, the minor compounds present in the extracts such as flavonoids can control this activity, in fact, *Bouchouk Soummam* and *Aghenfas* have higher activity (61.77 and 60.15%) than the *Ferkani* (57.84%) and *Tabelout* (49.34%) varieties, despite their high total phenolic contents. This higher activity could be explained by the concentrations of flavonoids, in fact, *Bouchouk Soummam* and *Aghenfas* varieties presented higher flavonoid contents and contain 41 and 44 $mg \cdot kg^{-1}$, respectively, corresponding to 26.03 and 23.71% of the total polyphenols, while the *Ferkani* and *Tabelout* varieties contain 13 and 14 $mg \cdot kg^{-1}$, respectively, corresponding to 4.87 and 4.11% of the total polyphenols. The abilities of the flavonoids to scavenge the radicals were related the presence of functional groups in their structure, mainly the B-ring catechol, the 2,3-double bond conjugated with the 4-oxo function. Luteolin represents 72.7 and 70.7% of total flavonoids in extracts from *Aghenfas* and *Bouchouk Soummam,* respectively; this compound with two hydroxyl in the B-ring is several times stronger than apigenin (Majewska *et al.*, 2011).

#### *3.4.2. ABTS.$^+$ assay*

All the samples tested were able to scavenge the $ABTS^{\cdot+}$ radical cation. Table 3 shows that the results were statistically different among the olive oil varieties ($p<0.05$). The results are also expressed as $IC_{50}$, (Table 3), representing the concentration needed to decrease the initial $ABTS^{\cdot+}$ concentration by 50%, a smaller $IC_{50}$ value corresponds to a higher antioxidant activity of the extracted olive oil.

The best $ABTS^{\cdot+}$ scavenging activity was shown by the extract from the *Bouricha* variety (73.38%) followed by the *Blanquette de Guelma* variety (66.73%). They exhibited the lowest $IC_{50}$ values (0.12 $mg \cdot mL^{-1}$ and 0.18 $mg \cdot mL^{-1}$ respectively), corresponding to a higher efficiency of the oil extracts to neutralize the radical $ABTS^{\cdot+}$; whereas extracts from the *Limli, Akerma* and *Chemlal Tazmalt* varieties showed a lower $ABTS^{\cdot+}$ scavenging activity (46.25, 46.19 and 45.25%) and higher $IC_{50}$ values (0.33, 0.42, 0.37 $mg \cdot kg^{-1}$). A significant correlation between the polyphenol contents and the $ABTS^{\cdot+}$ scavenging ability ($r = 0.72$, $p < 0.05$) was found. This coefficient is very close to that noted by Minioti and Georgiou (2010). Despite its high content of phenolic compounds, the *Neb Djemel* variety presented a lower antioxidant activity of 61.20% ($IC_{50} = 0.25$ $mg \cdot mL^{-1}$) compared with other varieties such as *Bouricha* and

Table 3. Scavenging activity of different extracts Algerian olive oils determined by DPPH and ABTS.$^+$ methods

| | Scavenging activity | | |
|---|---|---|---|
| **Varieties** | **DPPH (%)** | **ABTS.$^+$ (%)** | **ABTS.$^+$ IC50 (mg mL–1)** |
| Aghenfas | $60.17 \pm 2.77^{cd}$ | $58.33 \pm 1.61^{c}$ | $0.280 \pm 0.010^{e}$ |
| Akerma | $38.20 \pm 1.01^{a}$ | $46.19 \pm 0.64^{a}$ | $0.420 \pm 0.010^{g}$ |
| Blanquette de Guelma | $72.20 \pm 2.19^{e}$ | $66.73 \pm 1.54^{d}$ | $0.180 \pm 0.010^{b}$ |
| Bouchouk Soummam | $61.77 \pm 0.92^{de}$ | $59.96 \pm 1.03^{c}$ | $0.270 \pm 0.007^{d}$ |
| Bouricha | $64.61 \pm 2.27^{d}$ | $73.38 \pm 0.16^{e}$ | $0.12 \pm 0.017^{a}$ |
| Chemlal | $62.53 \pm 0.42^{de}$ | $60.23 \pm 0.22^{c}$ | $0.260 \pm 0.006^{cd}$ |
| Chemlal Tazmalt | $36.57 \pm 1.71^{a}$ | $45.25 \pm 1.66^{a}$ | $0.370 \pm 0.011^{f}$ |
| Ferkani | $57.84 \pm 1.23^{c}$ | $57.64 \pm 0.03^{c}$ | $0.300 \pm 0.005^{f}$ |
| Limli | $39.96 \pm 1.79^{a}$ | $46.25 \pm 1.05^{a}$ | $0.330 \pm 0.005^{e}$ |
| Neb Djemel | $63.37 \pm 0.45^{de}$ | $61.21 \pm 1.64^{c}$ | $0.250 \pm 0.002^{c}$ |
| Tabelout | $49.34 \pm 1.71^{b}$ | $49.82 \pm 1.48^{b}$ | $0.320 \pm 0.005^{e}$ |

$^{a-f}$ Means the column followed by different letters are significantly different ($P<0.05$). (mean±SD, n=3).

Table 4. Antibacterial activity (Inhibition zone diameter (mm)) of Algerian olive oil extracts against each bacterial species.

| Varieties | Concentrations (mg·disc$^{-1}$) | Micro-organism Inhibition zone diameter (mm)• | | | | | |
|---|---|---|---|---|---|---|---|
| | | S. aureus | B. subtilis | L. innocua | E. coli | P. aeruginosa | K. pneumoniae |
| Aghenfas | 1 | 19.00±0.00$^{p}$ | 4.00±1.00$^{d}$ | – | – | – | – |
| | 0.5 | 17.66±0.57$^{no}$ | 2.66±0.57$^{c}$ | – | – | – | – |
| | 0.25 | 15.00±0.00$^{kl}$ | – | – | – | – | – |
| | 0.12 | 13.00±0.00$^{hij}$ | – | – | – | – | – |
| Akerma | 1 | 18.00±0.00$^{nop}$ | 03.00±0.00$^{cd}$ | – | – | – | – |
| | 0.5 | 17.33±1.15$^{mn}$ | 01.00±0.00$^{ab}$ | – | – | – | – |
| | 0.25 | 14.33±1.15$^{gk}$ | – | – | – | – | – |
| | 0.12 | 11.00±1,00$^{fg}$ | – | – | – | – | – |
| Blanquette | 1 | 21.33±0.57$^{q}$ | 06.33±0.57$^{ij}$ | 2.33±0.57$^{a}$ | 02.00±1.00$^{ab}$ | – | 04.00±1.00$^{b}$ |
| de Guelma | 0.5 | 17.33±0.57$^{mn}$ | 05.33±0.57$^{fg}$ | – | 00.66±0.57$^{a}$ | – | 03.33±0.57$^{b}$ |
| | 0.25 | 13.00±0.00$^{hij}$ | 04.00±0.00$^{de}$ | – | – | – | 02.66±0.57$^{b}$ |
| | 0.12 | 08.33±0.57$^{de}$ | 01.00±0.00$^{a}$ | – | – | – | 00.66±0.57$^{a}$ |
| Bouchouk | 1 | 10.33±0.57$^{f}$ | 04.00±0.00$^{de}$ | – | – | – | – |
| Soummam | 0.5 | 08.33±0.57$^{d}$ | 02.33±0.57$^{bc}$ | – | – | – | – |
| | 0.25 | 05.00±0.00$^{c}$ | 01.00±0.00$^{ab}$ | – | – | – | – |
| | 0.12 | 02.00±0.00$^{ab}$ | – | – | – | – | – |
| Bouricha | 1 | 26.00±0,00$^{s}$ | 11.00±0.81$^{l}$ | 09.66±0.47$^{c}$ | 08.66±0.94$^{d}$ | 09.00±0.00$^{d}$ | 10.66±0.47$^{e}$ |
| | 0.5 | 22.66±0.47$^{r}$ | 10.33±0.94$^{l}$ | 09.00±0.81$^{bc}$ | 07.66±0.94$^{d}$ | 07.66±0.47$^{c}$ | 08.00±0.81$^{d}$ |
| | 0.25 | 20.33±0.47$^{q}$ | 09.33±0.47$^{k}$ | 08.00±0.81$^{bc}$ | 07.00±0,00$^{c}$ | 07.00±0.00$^{b}$ | 07.00±0.00$^{cd}$ |
| | 0.12 | 17.00±0.00$^{mn}$ | 08.66±0.47$^{jk}$ | 07.33±0.47$^{b}$ | 06.00±0.81$^{b}$ | 06.00±0.00$^{a}$ | 06.00±0.00$^{c}$ |
| Chemlal | 1 | 16.00±0,00$^{lm}$ | 06.00±0.00$^{gh}$ | – | – | – | – |
| | 0.5 | 12.00±1.00$^{gh}$ | 04.33±0.57$^{e}$ | – | – | – | – |
| | 0.25 | 09.66±0,57$^{f}$ | 02.00±0.00$^{abc}$ | – | – | – | – |
| | 0.12 | 07.33±0.57$^{d}$ | 01.00±0.00$^{a}$ | – | – | – | – |
| Chemlal | 1 | 04.33±0.57$^{c}$ | – | – | – | – | – |
| Tazmalt | 0.5 | 03.00±0.00$^{b}$ | – | – | – | – | – |
| | 0.25 | 01.00±0.00$^{a}$ | – | – | – | – | – |
| | 0.12 | – | – | – | – | – | – |
| | 1 | 14.66±0.57$^{k}$ | 06.66±0.57$^{h}$ | – | – | – | – |
| Ferkani | 0.5 | 09.66±0.57$^{f}$ | 04.66±0.57$^{ef}$ | – | – | – | – |
| | 0.25 | 04.66±0.57$^{c}$ | 02.00±0.00$^{ab}$ | – | – | – | – |
| | 0.12 | 02.66±0.57$^{b}$ | 01.00±0.00$^{a}$ | – | –- | – | – |
| | 1 | 12.66±0.57$^{hi}$ | 2.33±00.57$^{bc}$ | – | – | – | – |
| Limli | 0.5 | 07.66±0.57$^{d}$ | 01.00±0.00$^{ab}$ | – | – | – | – |
| | 0.25 | 05.66±0.57$^{c}$ | – | – | – | – | – |
| | 0.12 | 02.66±0.57$^{b}$ | – | – | – | – | – |
| | 1 | 19.00±0.00$^{p}$ | 08.66±0.57$^{i}$ | – | – | – | – |
| Neb Djemel | 0.5 | 17.33±0.57$^{mn}$ | 06.66±0.57$^{h}$ | – | – | – | – |
| | 0.25 | 14.00±1.00$^{ijk}$ | 04.00±0.00$^{de}$ | – | – | – | – |
| | 0.12 | 10.00±1.00$^{f}$ | 02.00±0.00$^{abc}$ | – | – | – | – |
| | 1 | 05.00±0.00$^{c}$ | – | – | – | – | – |
| Tabelout | 0.5 | 03.00±1.00$^{b}$ | – | – | – | – | – |
| | 0.25 | 01.66±0.57$^{ab}$ | – | – | – | – | – |
| | 0.12 | – | – | – | – | – | – |

Means (mean±DS, n=3) with the same letter in the same column are not significantly different. Diameter of zone of inhibition (mm) not including diameter of 6 mm disc;. –: No inhibitory effects.

*Blanquette de Guelma.* This could be explained by a higher content of free hydroxtyrosol and lignans of these two varieties. Literature data attests that hydroxytyrosol shows a high antioxidant activity (Lavelli, 2002). Regarding lignans' antioxidant power, Owen *et al.* (2000) showed the existence of a clear correlation between the radical scavenging ability of a virgin olive oil phenolic extract and the lignan concentration. The antioxidant activity of (+)-pinoresinol was more prominent that the activity of the following compounds: ligstroside aglycon, tyrosol, elenolic acid (Carrasco-Pancorbo *et al.*, 2005).

### 3.5. Antibacterial Activity

The different olive oils extracts at different concentrations (0.12, 0.25, 0.5 and 1 mg·disc$^{-1}$) were screened for their antimicrobial activity against *B. subtilis, S. aureus* and *L. innocua* (Gram-positive), *E. coli, P. aeruginosa, and K. pneumoniae* (Gram-negative) following the agar diffusion method (Table 4). The minimal inhibitory concentration (MIC) values for bacteria (Table 5) were determined. Standard antibiotics (Gentamycin, Chloramphenicol and Ofloxacine) were used, and are also mentioned in Table 6. The solvents used for extraction were also used for dissolving the extracts, and all the solvent controls did not show any activity (data not shown).

As shown in Table 4, the results of the in-vitro antimicrobial activity assay showed that the phenolic extracts from the *Bouricha* and *Blanquette de Guelma* varieties possessed broad antibacterial activity against the microorganisms tested. The zone of inhibition varied from 6 to 26 mm and from 0.6 to 21 mm, respectively. The extracts from the *Chemlal Tazmalt* and *Tabelout* varieties only showed activity against *S. aureus*. Whereas the others eight extracts displayed a moderate activity against *S. aureus* and *B. subtilis.* Our results are in concordance with those reported by Medina *et al.* (2006) who observed higher activity in virgin olive oils and none in other edible vegetable oils (corn, sunflower, soybean, rapeseed and cotton). The authors attributed this activity of olive oil to the phenolic compounds; the dialdehidic form of decarboxymethyl oleuropein and ligstroside aglycons, hydroxytyrosol, and tyrosol were the phenolic compounds that statistically correlated with bacterial survival. A recent study by Karaosmanoglu *et al.* (2010) showed different antimicrobial activities among extra virgin olive oil, refined olive oil and canola oil against three microorganisms (*E. coli, S. enteritidis and L. monocytogenes*).This activity was also related to the high concentrations of phenolic compounds found in virgin olive oils. The oil contained many phenolic compounds; an increase in their overall effect can be related to a synergistic interaction.

The effect of each individual phenolic compound, isolated by HPLC, against *L. monocytogenes,* showed that the dialdehydic form of decarboxy methyligstroside presented a potent antimicrobial activity. These results are in agreement with ours because the Algerian olive oil varieties presented high percentages of oleuropein and ligtroside derivatives.

Gentamycine, chloramphenicol and ofloxacine, which were used as a positive control, had inhibition zones ranging from 28 to 38 mm. *S. aureus*, with high sensitivity to some extracts, was also sensitive to antibiotics at a concentration much lower than methanolic extracts.

The MICs reported in Table 6 show quantitative evidence of the antimicrobial activity of different extracts the olive oil varieties. The MICs of the extracts varied from 0.6 to 2 mg·mL$^{-1}$ on most of the tested bacteria. In fact, the growth of *S.aureus* was significantly inhibited (0.6-1.6 mg·mL$^{-1}$). The lowest MIC value (0.6 mg·mL$^{-1}$) was observed for the extract from *Bouricha* on *S.aureus*. These values of MIC are higher than those reported by Pereira *et al.* (2006) for the phenolic compound extract from Portuguese table olives. Nevertheless, they are in accordance with the results reported by Tunçel and Nergis (1993) on the MIC of ferulic, vanillic, and caffeic acids and tyrosol against S. aureus, which were determined as 0.6, 0.55, 0.4 and 0.6 mg.mL$^{-1}$, respectively, by the agar dilution method.

The MICs of phenolic compounds that inhibit bacterial growth are much higher than those found in olive oils. In the study by Bisignano et al. (1999), minimum inhibitory concentrations of hydroxytyrosol against *Staphylococcus aureus* (penicillin susceptible) and *Staphylococcus aureus* (penicillin-resistant) were determined; they ranged from 3.9–31.25 μg.mL$^{-1}$ and 3.9–31.25 μg.mL$^{-1}$, respectively. These concentrations of this phenolic compound

Table 5. Antibacterial activity (Inhibition zone diameter) of antibiotics against each bacterial species.

| | Concentrations (μg·disc$^{-1}$) | Microorganisms Inhibition zone diameter (mm)• | | | | | |
|---|---|---|---|---|---|---|---|
| | | S. aureus | B. subtilis | L. innocua | E. coli | P. aeruginosa | K. pneumoniae |
| Gentamycin | 15 | 30.7±1.2 | 34.0±0.0 | 26.7±1.2 | 28.7±1.2 | 26.0±0.0 | – |
| Chloramphenicol | 30 | 30.7±1.2 | 34.7±1.2 | 31.3±1.2 | 34.7±1.2 | 22.7±1.2 | – |
| Ofloxacin | 5 | 32.0±0.0 | 36.7±1.2 | 38.0±0.0 | – | 38.0±2.0 | 30.0±0,0 |

Diameter of zone of inhibition (mm) including diameter of 6 mm disc; –: No inhibitory effects (mean±SD, n=3).

Table 6. Minimum Inhibitory Concentration (MIC) of different extracts of Algerian olive oils against Each Bacterial Species

| | MIC mg·mL$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | **S. aureus** | **B. subtilis** | **L. innocua** | **P.aeruginosa** | **E. coli** | **K. pneumoniae** |
| Aghenfas | 0.9 | 1.3 | NT | NT | NT | NT |
| Akerma | 0.9 | 1.4 | NT | NT | NT | NT |
| Blanquette de Guelma | 0.7 | 1.2 | 1.8 | NT | 1.8 | >2 |
| Bouchouk Soummam | 1.2 | 2 | NT | NT | NT | NT |
| Bouricha | 0.6 | 1.2 | 1.6 | 1/1.1 | 1.8 | >2 |
| Chemlal | 1.2 | 1.2 | NT | NT | NT | NT |
| Chemlal Tazmalt | 1.6 | NT | NT | NT | NT | NT |
| Ferkani | 1 | 1.2 | NT | NT | NT | NT |
| Limli | 1.2 | 1.8 | NT | NT | NT | NT |
| Neb Djemel | 0.9 | 1.2 | NT | NT | NT | NT |
| Tabelout | 1.6 | NT | NT | NT | NT | NT |

NT= Not tested

are about 1000 times higher than the levels of those in extra virgin olive oil, EVOO.

Among the bacteria used in the present study, *S. aureus* (Gram-positive) showed a higher sensitivity to different extracts with the largest zone of inhibition and lowest MIC (Tables 4 and 6). This was consistent with the results from previous reports, which suggested a higher susceptibility of food-borne pathogenic Gram-positive bacteria to polyphenols compared with Gram-negative bacteria (Brenes *et al*., 2006). This observation can be attributed to differences in the structure of the bacteria cell wall. The less complex the structure of the cell wall is in the Gram-positive bacteria, the more permeable it is to anti-microbial compounds (Papadopoulou *et al*., 2005).

Although the anti-microbial effects of various phenolic extracts are well documented, the mechanisms of action of these products and their components are not fully understood. Polyphenols are thought to cause surface activity that damages the membranes of bacterial cells, to inhibit its enzymes, or to interfere with the production of certain amino acids necessary for bacterial growth (Cowan, 1999). 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 relative 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 oleuropein might render the unable to penetrate cell membranes or to reach the target site.

## 4. CONCLUSIONS

The phenolic composition represents a useful contribution to the biochemical characterization of the Algerian olive oil cultivars. Derivatives of oleuropein and ligstroside, the abundant phenolic compounds hydroxytyrosol, tyrosol, flavonoids, and lignans vary quantitatively according to the variety; in fact, the cultivar affects quantitative phenolic factions of these EVOOS, demonstrating a unique and characteristic phenolic profile. These phenolic fractions also influenced and allowed to differentiate the total antioxidant and antibacterial activity observed in the cultivars.

The results obtained in the present work reveal that Algerian olive oils may constitute a good source of healthy compounds in the diet, especially phenolic compounds, suggesting that their consumption could be useful in the prevention of diseases in which free radicals are implicated and may be good candidates for applications such as antimicrobial agents against bacteria responsible for human gastro-intestinal and respiratory tract infections and foodborne pathogens, particularly important against *S. aureus*, a microorganism extensively studied due to its ability to produce enterotoxins. As far as we know, this is the first report considering the antioxidant and anti-microbial potential of phenolic compounds in EVOO from Algeria.

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