Phytothérapie
DOI 10.3166/phyto-2019-0199

PHARMACOGNOSIE

Antioxidant, Antibacterial and Antifungal Effects of Phenolic Extracts of Extra Virgin Olive Oil from Two Western Regions of Algeria: A Comparative Study

Effets antioxydants, antibactériens et antifongiques d'extraits polyphénoliques d'huile d'olive extravierge de deux régions de l'Ouest algérien : une étude comparative

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Abstract The aim of the study was to evaluate the antioxidant, antibacterial and antifungal activities of phenolic extracts of extra virgin olive oil obtained from two distinct regions in Northwest Algeria. The first extra virgin oil (CHIALI) was produced industrially according to the international standards, whereas the second (TRAD) was produced by traditional methods. Antioxidant activity was evaluated using the DPPH (diphenylpicrylhydrazine) method. The antimicrobial activity of the two phenolic extracts was assessed against *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923), *Alternaria* sp., *Candida albicans* by using microdilution and disk diffusion methods. The extraction yield was found to be 26.7% and 20.5% for the industrial (CHIALI) and traditional methods (TRAD), respectively. The phenolic extract of the industrial extra virgin oil exhibited better antioxidant activity (IC_{50} = 1.56 µg/ml) when compared to those of the traditional oil (IC_{50} = 6.27 µg/ml). Both phenolic extracts exerted bactericidal and antifungal activities. These activities were more important with the phenolic extract of the industrial oil. Taken together, our results showed that the phenolic extract of the industrial extra virgin oil had better antioxidant, antibacterial and antifungal activities, owing to the extraction methods used.

Keywords Extra virgin olive oil · Polyphenolic · Antioxidant · Antibacterial · Antifungal

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Résumé Le but de cette étude a été d'évaluer les activités antioxydantes, antibactériennes et antifongiques d'extraits phénoliques d'huile d'olive extravierge obtenue de deux régions distinctes du Nord-Ouest algérien. La première huile d'olive extravierge (CHIALI) a été produite industriellement selon les règles internationales tandis que la seconde (TRAD) l'a été par des méthodes traditionnelles. L'activité antioxydante a été évaluée par la méthode au DPPH (diphénylpicrylhydrazine). L'activité antimicrobienne des deux extraits phénoliques a été évaluée contre *Escherischia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923), *Alternaria* sp, *Candida albicans* en utilisant les méthodes de microdilution et de disques de diffusion. Nous avons trouvé comme rendement d'extraction, respectivement, 26,7 % pour la méthode industrielle (CHIALI) et 20,5 % pour la traditionnelle. L'extrait phénolique de l'huile extravierge industrielle montre une meilleure activité antioxydante (IC_{50} = 1,56 µg/ml) en comparaison de celle de l'huile traditionnelle (IC_{50} = 6,27 µg/ml). Les deux extraits phénoliques présentent des activités antibactériennes et antifongiques. Ces activités sont plus importantes avec l'extrait phénolique de l'huile industrielle. Tout considéré, nos résultats montrent que l'extrait phénolique de l'huile extravierge industrielle a de meilleures activités antioxydantes, antibactériennes et antifongiques en rapport avec la méthode d'extraction utilisée.

Mots clés Huile d'olive extravierge · Polyphénolique · Antioxydant · Antibactérien · Antifongique

Introduction

The Mediterranean diet has shown its benefits to the health of consumers, partly owing to the consumption of virgin olive oil by people living in the Mediterranean basin [1].

Due to its high antioxidant content, such as polyphenols (hydroxytyrosol and tyrosol), olive oil contributes to the prevention and treatment of cardiovascular diseases, cancers, diabetes, neurodegenerative diseases, inflammation and aging. In addition, they are characterized by an antimicrobial capacity as they enhance the immunity and protection of certain tissues and organs against oxidative damage [2]. Indeed, several studies have demonstrated the antimicrobial potency of polyphenols in olive oil against a broad spectrum of pathogenic microbes [3,4]. Oleuropein and hydroxytyrosol have been reported to inhibit or slow the growth of different bacteria [5]. Due to the emergence of bacterial resistance to antibiotics and especially the emergence of multidrug-resistant bacteria in Algeria, the control and management of bacterial infections is becoming more complex [6]. It is in this context that the development of new therapeutic agents is essential and urgent.

Several factors condition the phenolic content of olive oil. These factors include variety, climatic conditions, degree of olive maturation, and oil extraction technology [7].

Algerian virgin olive oils have been shown to have significant antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* [8]. This bactericidal activity was attributed to the presence of phenolic compounds [9]. Indeed, olive oil is a substrate that does not promote survival and microbial growth. Only a few microorganisms use the fatty acids in olive oil as a source of carbon and energy and survive in the presence of its antimicrobial components [10].

The present study aims to evaluate the antioxidant, antibacterial, and antifungal activities of polyphenols of extra olive oil obtained from two distinct regions in Northwest Algeria (Sidi-Bel-Abbès and Mascara).

Material and methods

Extra virgin olive oil samples

The extra virgin olive oil samples were procured from two regions in the Northwest Algeria. The first extra virgin oil (CHIALI) was produced industrially according to the international standards, using two olive varieties: Chemlal and Sigoise growing in Sidi Belabbes. The second (TRAD) was produced by traditional methods using the Sigoise variety growing in Mascara.

Phenolic extract preparation

The phenolic compounds were extracted from the extra virgin olive oils as previously described by Gutfinger [11]. In brief, 50 ml of hexane are added to 50 g of olive oil. The mixture is vortexed at 300 rpm for 10 min, then 30 ml of a methanol–water solution (80/20, v/v) is added. After centrifugation at 6,000 rpm for 15 min, the lower phase is recovered. The operation is repeated three times; the pellets are combined and concentrated under vacuum until a dark yellow deposit of polyphenols is obtained.

The extraction yield (expressed as % [g of extract/100 g of olive oil]) was calculated using the following formula:

Yield (w/w %) = (M′/M) × 100

M′: weight of extract obtained (g)

M: Mass of the analyzed oil (g)

Antioxidant activity (DPPH assay)

The antioxidant activities of the phenolic extracts were evaluated by using the DPPH assay. 1 ml of the extracts prepared at different concentrations (1.25, 2.5, 5, 10, and 20 µg/ml) was mixed with 4 ml of a DPPH solution (prepared by dissolving of 1.95 mg of DPPH in 200 ml of methanol). The mixture was then placed in dark for 30 min. Absorbance was determined at 517 nm against a blank (pure methanol). Ascorbic acid was used as a positive control.

The inhibition percentage for scavenging DPPH radical was calculated according to the following formula: [(Ac − As)/Ac] × 100 where Ac is the absorbance of the reaction mixture without extract and As is the absorbance of the reaction mixture with sample.

Antimicrobial activity

Microbial strains

The antimicrobial activity assessment was carried out against five pathogen strains: *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923), *Alternaria* sp., and *Candida albicans*.

Antibacterial activity

The antimicrobial activity of the polyphenolic extracts was evaluated by the diffusion method on agar medium. This method is based on the competition between the growth of a bacterium and the diffusion of an antibiotic in an agar medium from a pre-impregnated paper support. After incubation, the diameter of the clear inhibition zone surrounding the disc will be proportional to the antimicrobial activity of the substances tested [12].

To optimize the bacterial strains' growth, they were seeded in nutrient agar and incubated at 37 °C for 24 h. The protocol followed was that reported by Kappel et al. [13]. Briefly, from a 24 h pure and fresh culture, a bacterial suspension (isolated colonies placed in 9 ml of physiological saline and homogenized) was prepared. The inoculas were adjusted until an optical density of 0.50 was obtained.

The bacterial suspension (10^7 CFU/ml) was seeded in Mueller-Hinton agar previously poured into Petri dishes. 6 mm sterile paper disks were each impregnated with 20 µl of an extract solution prepared in methanol/water (80/20) at the following dilutions (stock solution, 1/2, 1/4, 1/8 corresponding, respectively, to 1, 0.5, 0.25, 0.12 mg/disc). The methanol/water solution (80/20) without extracts was used as a negative control. After 2 h at 4 °C (to allow the diffusion of the extracts), the Petri dishes were incubated at 37 °C for 24 h, and the diameter of the inhibition zones were measured.

Antifungal activity

The antifungal activity of the phenolic extracts was evaluated based on the disc diffusion method. We followed the same steps of the study of the antibacterial activity. After an activation of the strains for 7 days at 28 °C, Sabouraud's dextrose agar plates were inoculated with prepared suspension. The diameters of growth inhibition zones were measured after 72 h incubation at 28 °C.

Results and discussion

Extraction of phenolic compounds

The extraction yield and characteristics of the phenolic extracts obtained are shown in table 1.

According to our results, the extra virgin oil obtained through industrial methods allowed a better extraction of phenolic compounds (yield = 26.7%), when compared to the traditional extraction (20.5%). This result shows an important difference between the two olive oils regarding their composition. Indeed, several studies have found important differences in the concentration of phenolic compounds in the olive oils obtained from different regions. Hrncirik and Fritsche [14] compared the concentration of total polyphenols in 23 samples of extra virgin olive oil from various countries (Italy, Spain, Greece, and Tunisia). They found a large variation in the concentration of total polyphenols.

Various factors may affect the polyphenolic profile in the olive oil such as: variety, climate, degree of ripening (green olives have higher concentrations of polyphenols than black olives) [15], temperature, and the extraction solvent (higher concentrations of polyphenols are obtained by using higher temperatures and a solvent consisting of 80% methanol) [16,17]. The polyphenolic content is not the same for all olive oils, hence its consideration as one of the quality criteria of these vegetable oils.

Table 1 Extraction yield and characteristics of the phenolic extracts

Extract	Aspect	Color	Yield (%)
CHIALI	Not viscous	Greenish yellow with a pungent smell of fresh olive	26.7
TRAD	Not viscous	Light green	20.5

The content of polyphenols in olive oil may vary depending on several parameters. It has been shown that the process of transformation (extraction) used is the first of these factors, and contributes to the distinction between virgin olive oil, extra virgin olive oil, or refined olive oil. Depending on the extraction process the polyphenol content may vary from 232 mg/kg in an extra virgin oil to 62 mg/kg in a refined oil.

Ortho-diphenols (hydroxytyrosol, caffeic acid, and oleuropein) passing in olive oil during its extraction are considered natural antioxidants that protect the oil against oxidation. They give it a better storage stability, a bitter taste and a piquant sensation [18,19].

Antioxidant activity

As shown in figure 1, both the phenolic extracts obtained from the CHIALI and TRAD extra virgin olive oils exhibited important scavenging activities. These activities were dose dependent. At 20 µg/ml, the TRAD and CHIALI phenolic extracts resulted in 98.5% and 91.9% of scavenging activity, respectively. The scavenging activity of the TRAD phenolic extract was comparable to that of the ascorbic acid (99.1%). At lower concentrations, the TRAD phenolic extract exerted promising scavenging activities, comparable to those of the ascorbic acid. On the other hand, at the same lower concentrations, the CHIALI phenolic extract showed scavenging activities, but less important than those obtained with the TRAD phenolic extract.

The antioxidant potential of polyphenols of the olive oil has been demonstrated [20,21]. Besides the inhibition of lipid peroxidation, phenolic compounds trap free radicals and therefore protect the human body [22]. The antioxidant effect of polyphenols is mediated through different pathways. In systems using the oxidation of transition metals such as copper and iron, they can chelate these metal ions. The latter are initiators of the Fenton reactions generating important concentrations of hydroxyl radicals [23]. On the other hand, the antiradical capacity seems to be the most important antioxidant activity of phenolic compounds by breaking the chain of reactions triggered by free radicals. The antioxidant property of polyphenols is associated with their ability to form intramolecular hydrogen bonds between the hydroxyl group and the phenoxylic radicals [24].

The scavenging activity of the phenolic extracts was then evaluated through calculating the IC_{50}. The IC_{50} corresponds to the concentration of polyphenols necessary to inhibit or reduce 50% of the DPPH. The lower the IC_{50}

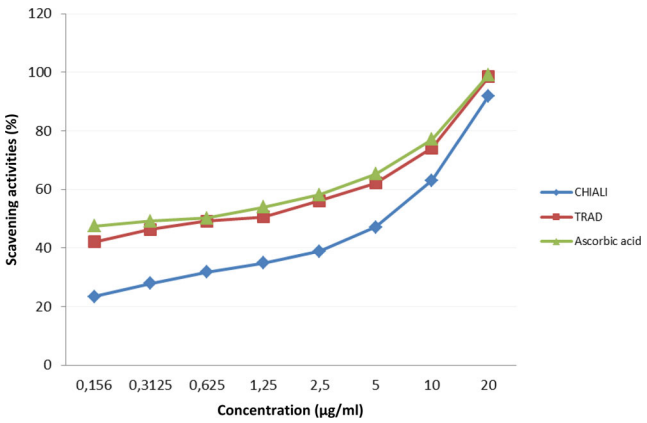

Fig. 1 DPPH free radical scavenging activity

value, the highest will be the free radical activity of the extract [25].

According to our results (Table 2), the phenolic extracts obtained from the CHIALI extra virgin oil possessed the best antioxidant activity (IC_{50} = 1.27 µg/ml), when compared to that of the phenolic extract from the TRAD oil (IC_{50} = 6.65 µg/ml).

Antimicrobial activity

Growth inhibition effect

In order to choose the solvent for the further experiments, we have compared the effect of DMSO and Tween 80 on the viability of the different microbial strains. As shown in table 3 and figure 2, only DMSO was safe regarding the microbial growth. Dimethyl sulfoxide (DMSO) is currently used to enhance the solubilization of polyphenolic extracts. With an inhibition diameter equal to that of the sterile disk, DMSO had no effect on the microbial strains at the concentrations used. Thus, we have adopted DMSO for dissolving the phenolic extracts in order to assess their antimicrobial activities.

Table 2 IC_{50} values of DPPH-scavenging activity of phenolic extracts

	Ascorbic acid	**TRAD**	**CHIALI**
IC_{50} (µg/ml)	0.006	6.65	1.27

The antimicrobial activities of both the CHIALI and TRAD phenolic extracts are given in table 4. The results represent the diameter of inhibition zone around each disc at the end of incubation period.

The results obtained show that both extracts resulted in an inhibition of the tested strains. This inhibition, as reflected by the inhibition diameter was dose-dependent. According to Mutai et al. [26] the diameters of inhibition zones (D) of the microbial growth are classified as follows: very strongly inhibitory ($D \geq 30$ mm), highly inhibitory (21 mm $\leq D \leq$ 29 mm), moderately inhibitory (16 mm $\leq D \leq$ 20 mm), slightly inhibiting (11 mm $\leq D \leq$ 16 mm) and noninhibiting ($D \leq 10$ mm).

At 500 µg/ml, both phenolic extracts (CHIALI and TRAD) resulted in the growth inhibition of the five tested strains. *Staphylococcus aureus* was the most sensitive to the antiproliferative effect of the phenolic extracts CHIALI and TRAD with an inhibition diameter of 30.43 and 21.03 mm, respectively. *Candida albicans* was also sensitive to the CHIALI and TRAD phenolic extracts with an inhibition diameter of 22.33 and 19.52 mm, respectively, followed by *Pseudomonas aeruginosa* (22.23 and 19.05 mm), *Escherichia coli* (19.32 and 16.23 mm), and *Alternaria* sp. (17.78 and 14.74 mm).

Taken together, our results reveal that the phenolic extracts of both CHIALI and TRAD extra virgin olive oil exert growth-inhibitory activities against five microbial strains involved in the food toxi-infections.

Minimum inhibitory concentrations

The antimicrobial effect and the minimum inhibitory concentrations of the TRAD and CHIALI phenolic extracts are showed in table 5.

According to the results obtained (Table 5), *Staphylococcus aureus* was found to be the most sensitive strain at the different concentrations (500, 250, 125 µg/ml) of CHIALI phenolic extracts. Similarly, at 250 and 500 µg/ml, this extract inhibited the growth of both *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Alternaria* sp. Regarding the antimicrobial effect of the phenolic extract of the TRAD extra virgin oil, our results showed that the *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* were the most sensitive strains toward the concentration of 500 µg/ml. Moreover, *Escherichia coli*,

Table 3 Effect of DMSO and Tween 80 on the microbial growth

Strains / **Solvent**	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Alternaria sp.***	***Candida albicans***
DMSO	−	−	−	−	−
Tween 80	+	+	+	+	+

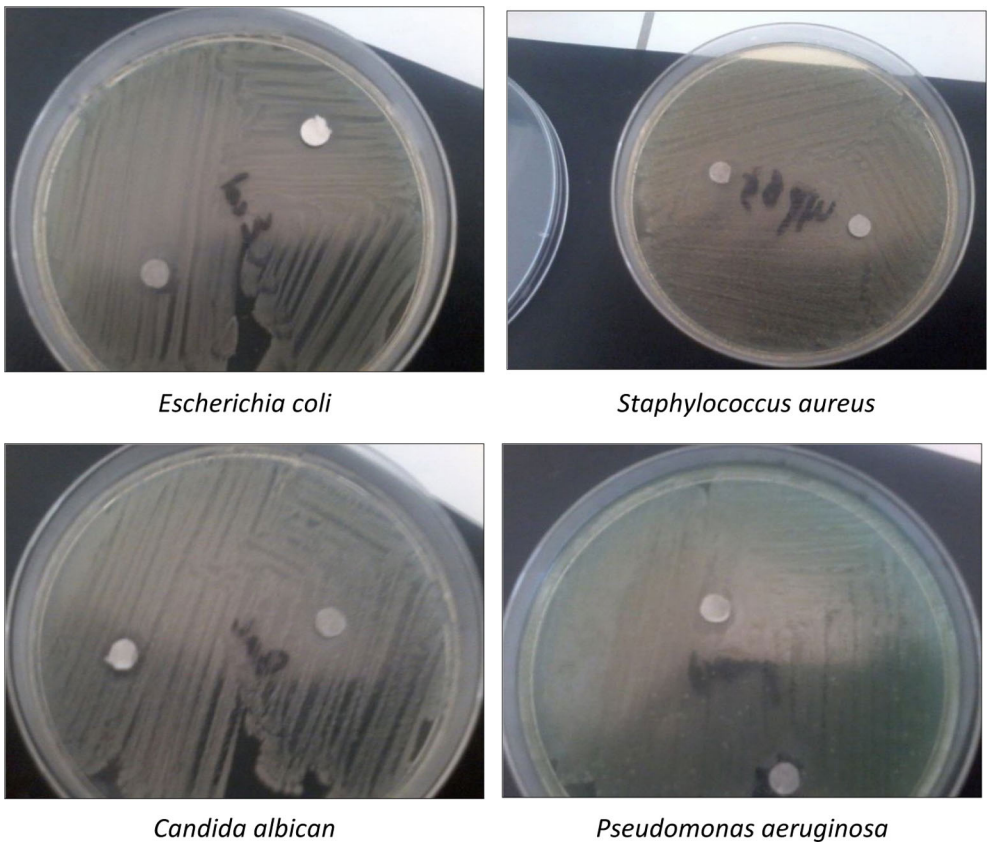

Fig. 2 Effect of the DMSO on the microbial growth

Table 4 Antimicrobial activity of the phenolic extracts. Mean inhibition diameter

Bacterial strains and fungal strains		***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Candida albicans***	***Alternaria* sp.**
Sample	**Concentration (µg/ml)**	**Inhibition diameter (mm)**				
CHIALI	500	19.32	30.43	22.23	22.33	17.78
	250	13.63	20.25	17.57	19.43	14.33
	125	12.60	14.04	13.09	15.25	12.90
	62.5	10.94	11.25	11.11	13.60	11.28
	31.25	7.32	9.11	9.34	9.65	8.05
TRAD	500	16.23	21.03	19.05	19.52	14.73
	250	11.37	16.12	15.36	13.31	13.82
	125	10.09	9.05	9.98	11.98	11.27
	62.5	9.89	8.13	8.65	10.34	9.12
	31.25	6.50	7.25	5.11	9.02	7.88

Pseudomonas aeruginosa, and *Alternaria* sp. had a mean sensitivity to the TRAD phenolic extract at 125 µg/ml.

Our findings are in agreement with those previously published. Indeed, Medina et al. [3] demonstrated that the olive oil possessed a stronger bactericidal effect against harmful bacteria to the intestinal flora (*Clostridium perfringens* and *Escherichia coli*), when compared to other vegetable oils (sunflower, colza, soy, etc.). They reported that food-borne pathogenic bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia sp*., and

Table 5 Antimicrobial activities and MIC (µg/ml) of TRAD and CHIALI phenolic extracts

Strain	Phenolic extract	Concentrations (µg/ml)					
		Control	1/32 31.25	1/16 62.5	1/8 125	1/4 250	1/2 500
Escherichia coli	CHIALI	−	−	− MIC	+	+	+++
	TRAD	−	−	−	− MIC	+	+++
Staphylococcus aureus	CHIALI	−	−	− MIC	+++	+++	+++
	TRAD	−	−	−	−	− MIC	+++
Pseudomonas aeruginosa	CHIALI	−	−	− MIC	+	+++	+++
	TRAD	−	−	−	−	− MIC	+
***Alternaria* sp.**	CHIALI	−	−	− MIC	+	+++	+++
	TRAD	−	−	−	− MIC	+	+
Candida albicans	CHIALI	−	−	− MIC	+	+++	+++
	TRAD	−	−	− MIC	+	+	+++

(+++): High growth, (+): Low growth; (−): no growth

Shigella sonnei) did not survive after 1 h of incubation in olive oil. Furthermore, several studies have shown that the extra virgin olive oil polyphenols were able to retard or inhibit the growth of different bacteria and fungi strains including those considered pathogenic to humans. Also, it has been reported that the vegetation water resulting from the olive oil extraction was toxic to the phytopathogenic bacteria *Pseudomonas syringae* (Gram-negative) and *Corynebacterium michiganense* (Gram-positive). In the same context, Capasso et al. [27] found that among the main polyphenols of the vegetation water, methylcatechol exhibited a strong bactericidal activity against *Pseudomonas syringae* and was slightly active against *Corynebacterium michiganense*. Other polyphenols, such as catechol and hydroxytyrosol, were less active against *Pseudomonas syringae*, and inactive against *Corynebacterium michiganense*. Bisignano et al. [28] evaluated the susceptibility of several human respiratory and intestinal pathogens to hydroxytyrosol and oleuropein. The pathogens studied were five standard bacterial strains (*Haemophilus influenzae* ATCC9006, *Moraxella catarrhalis* ATCC8176, *Salmonella typhi* ATCC6539, *Vibrio parahaemolyticus* ATCC17802 and *Staphylococcus aureus* ATCC25923) and 44 fresh isolates (isolated from patients) including 8 strains of *Haemophilus influenza*, 6 strains of *Moraxella catarrhalis*, 15 strains of *Salmonella typhi*, 1 strain of *Vibrio cholerae*, 2 strains of *Vibrio alginolyticus*, 1 strain of *Vibrio parahaemolyticus*, and 11 strains of *Staphylococcus aureus* (5 penicillin-sensitive and 6 penicillin-resistant strains). This study demonstrated an important antibacterial activity of hydroxytyrosol against the different strains tested, with an MIC of 0.24–7.85 mg/ml against the standard strains and 0.97–31.25 mg/ml against the isolated strains. The study concluded that hydroxytyrosol could be used in the treatment of bacterial infections of the intestinal and respiratory tract in humans.

In the same line with our findings, it has been found that the phenolic extracts of extra virgin olive oil inhibited *Candida albicans* growth [29]. These phenolic extracts were shown to possess a bactericidal effect against *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*. This effect was considered to be stronger that of the synthesized disinfectants glutaraldehyde and ortho-phthalaldehyde [30].

In addition, polyphenols present in extra virgin olive oil have been shown to contribute to the preservation of certain food products such as yogurts and mayonnaise [31].

Conclusion

The present study compared phenolic compounds extracted from two extra virgin olive oils and their activities. Our results showed that the extra-virgin olive oil produced industrially according to international standards had a higher percentage of phenolic products, in comparison with that produced traditionally. Besides, the phenolic extract of the industrial extra virgin oil exhibited better antioxidant activity. On the other hand, the study of the antimicrobial and antifungal activity showed that the phenolic extracts of extra virgin olive oil (industrial and traditional) had a significant antimicrobial activity. Both phenolic extracts appear to have anti-*Escherichia coli*, anti-*Pseudomonas*, anti-*Streptococcus aureus*, and antifungal activity.

Conflicts of interests: the authors have no conflicts of interests to declare.

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