Oleuropein and Antibacterial Activities of Olea europaea L. Leaf Extract

Himour S.
Laboratory Science Natural and Materials.
University Mentouri Constantine.
Yahia A.
Belattar H.
University Centre Abdelhafid Boussouf. Mila.

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Abstract

In this study, we reported the determination of phenolic compounds in olive leaves by reversed phase HPLC/DAD and the evaluation of their *in vitro* activity against several microorganisms. These organisms might however, be causal agents of human intestinal and respiratory tract infections. Extract of the leaves of two varieties of *Olea europaea* L. (*Chemlel* and *Dathier*) was investigated for antibacterial activity against four pathogenic bacteria.
Leaves extract was prepared using water and methanol (20/80) in a cold extraction process. The tested bacteria were ***Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus*** .The extracts were found to be effective against all isolates tests. Ethanolic extract at a concentration of 100 % presented the highest potential of inhibiting variety of *Dathier* against *S.aureus* .This is with an inhibition zone of 17.49 mm and 15.66 mm for the variety *Chemlel* against *S.aureus.* The high Oleuropein content and the important antibacterial activities of olive leaves extract could be useful sources for industrial extraction and pharmacological application.

Keywords: Antibacterial activity, *Olea europaea*, Oleuropein, HPLC

Introduction

Antibiotics are one of the most important weapons in fighting bacterial infections. It has greatly benefited the human's health since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses. This is not only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Traditional medicine has

remained the most affordable and easily accessible source of treatment in the primary health care system of poor communities (**Hosseinzadeh et *al*., 2015).**

Subsequently, the antimicrobial activity of a plant is highly related to secondary substances that are synthesized and produced by these plants. Secondary metabolites are substances of low molecular weight, which were not the products of the primary metabolic pathway of the producing organism. However, it was first thought to be of no advantage to the plant. Nowadays, it has been believed that they have vital functions (**Kant et *al*., 2010**).

Olea europaea preparations have been used widely in folk medicine in Mediterranean area. Olive leaf is one of the potent source of plant polyphenols having antioxidant, antimicrobial, and antiviral properties due to its rich phenolic content (**Aytul, 2010**).

The large number of phenolic compounds present in olive leaves aroused the interest of researchers around the world. Also, the studies with animals and humans have reported beneficial health effects such as the capacity of antioxidant, anti-hypertensive, hipo-glicemiant, hypocholesterol, anti-inflammatory, and as co adjuvant in the treatment of obesity (**Esmaeili-Mahani et *al*., 2010**).

The objectives of this work entail the determination of Oleuropein in the leaves of *Chemlel and Dathier*. Also, it aims to test the extract of *Olea europaea* for antibacterial activity .

Materials and Methods

Plant Material

Olea europaea leaves specimens are collected every autumn between October and November. The leaves we used in this experiment were collected in November 2014 from the region of Mila in the North East of Algeria. However, it was collected exactly from Maazouzi Lekhder station. The leaves were dried for twenty days at the ambient laboratory temperature (20-30°C).

They were milled to a fine powder in an electrical mill and stored in the dark at laboratory temperature in closed containers until required.

Preparation of *Olea europaea* Leaves Extract

The resulting powder is kept away from air, moisture, and light in tightly closed glass vials. To extract the polyphenols total, we took 5 g of each powder of olive tree leaves and macerate in100 ml methanol for five days. After stirring, the recovered filtrate was dried in a rotary evaporator for 20 minutes at 60° C.

However, the polyphenols are then rest orated with 10ml of methanol (**Arab et *al*., 2013**).

Determination of Total Polyphenol Content

A diluted sample extract (2 ml) or phenolic standard was mixed with the Folin Denis reagent (2.5 ml) and aqueous sodium carbonate 20% (5 ml), in a 50 ml-volumetric flask. After 45 min, the volume constitutes water. Also, the total phenolic content was determined calorimetrically at 760 nm. The total phenolic content was expressed as mg of Gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g) through the calibration curve of Gallic acid (R2 = 0.99). The sample was analyzed in three replications (**Singelton et *al*., 1999**).

Determination of Total Flavonoids

The total flavonoids content of the leaf extract was determined according to the colorimetric assay developed by **Zhishen et *al*. (1999**).The total flavonoids content was calculated from the standard curve of Quercetin (10 - 250 mg. L^{-1}) plotted by using the same procedure. Hence, total flavonoids were expressed as mg Quercetin equivalents per gram of dried leaves.

HPLC Analysis of Olive Leaves Extracts

The HPLC analysis given in the literature was used for the identification and quantification of polyphenol compound (**Savournin et *al*., 2001**).

Consequently, HPLC analysis was performed on a SHIMADZU apparatus, manual injection valve, and UV detector model SPD20A. This was done using a column of 250 x 4.60 mm with per column, packing: Thermo Scientific ODS HYPERSIL C18. Furthermore data acquisition and quantitation were performed with SHIMADZU. The mobile phase was 92% distilled water acidified (pH=3) with 0.10 M orthophosphoric acid (v/v, 1000 : 2.30) and 21% acetonitrile (Carlo Erba, HPLC grade) acidified with 0.10 M orthophosphoric acid (v/v, 1000 : 2.30). The flow rate was 1 ml/min, and the injection volume was 20 µl. Routine quantitation of Oleuropein was assessed at 280 nm and run time was 8 min.

Determination of Antibacterial Activity of the Leaves Extract

All the bacteria tested (*E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*) were grown on Mueller Hinton Agar. The antibacterial activity of the extract was measured by diffusion test on Muelle–Hinton agar. However, this was previously inoculated with 1 ml of bacterial suspension reactivated by culture during 18 h with microbial charge of (106 CFU/Ml). Sterilized paper discs (6 mm) were impregnated with 20 µl of different concentrations of extract (100%, 50%, 25%) prepared in DMSO, and placed into nutrient agar. The plates were incubated at 4 ^{0}C for 2 h to allow

the diffusion of the active compounds in the medium. Negative controls were prepared using the same solvent employed to dissolve the plant extract. However gentamicin discs (10 µg, Oxoid, UK) had positive controls.

Plates incubation was performed at 37 ^{0}C for 24 h. Inhibition zones in mm (without disc paper diameter) around discs were measured. The antibacterial activity was expressed by the diameter of the inhibition zones produced by the extract against microorganisms which was tested (**Tagg & Mcgiven, 1971; Bouderba et *al.*, 2012).**

Result and Discussion

The evaluation of yields of extracts reveals the following order, *Dathier*, higher than *Chemlal* .This difference was probably related to genetic feature of each variety since their growth is in the same climatic and geographical conditions. In addition the extraction was carried out with the same solvent. The total phenol (TP) and flavonoid (TF) content of olive leaves obtained from two varieties are shown in Table 1 below.

Table 1. Yield of extraction, total phenols, and total flavonoïdes in olive leaves extracts.

Varieties	Extraction yields(%)	Total phénols (TP) (mgGAE/g)	Total flavonoïdes (TF) (mgCE/g)
Dathier	33	49.6	71.8
Chemlel	34.40	84.4	85.3

Table 1 reveals the extraction yields, total phenols, and total flavonoids. The total phenolic and total flavonoid contents of the leaves of two varieties *Dathier* (49.6mg GAE/g and 71.8 mg QE/g) and *Chemlel* (84.4mg GAE/g and 85.3 mg QE/g) respectively were significantly higher than the values obtained by **Abaza et *al*. (2011).** Furthermore, it is inferior than the values obtained by **Ben saleh et *al*. (2012**). Phenolic compounds are important leaves constituents because they exhibit antibacterial activity **(Michele Hansen et *al*., 2002)**

HPLC Analysis of the Olive Leaves Extracts

Analysis of the olive leaf extract by HPLC illustrated a complex mixture of phenolic compounds in the two varieties, *Chemle* and *Dathier* (Figure 1).

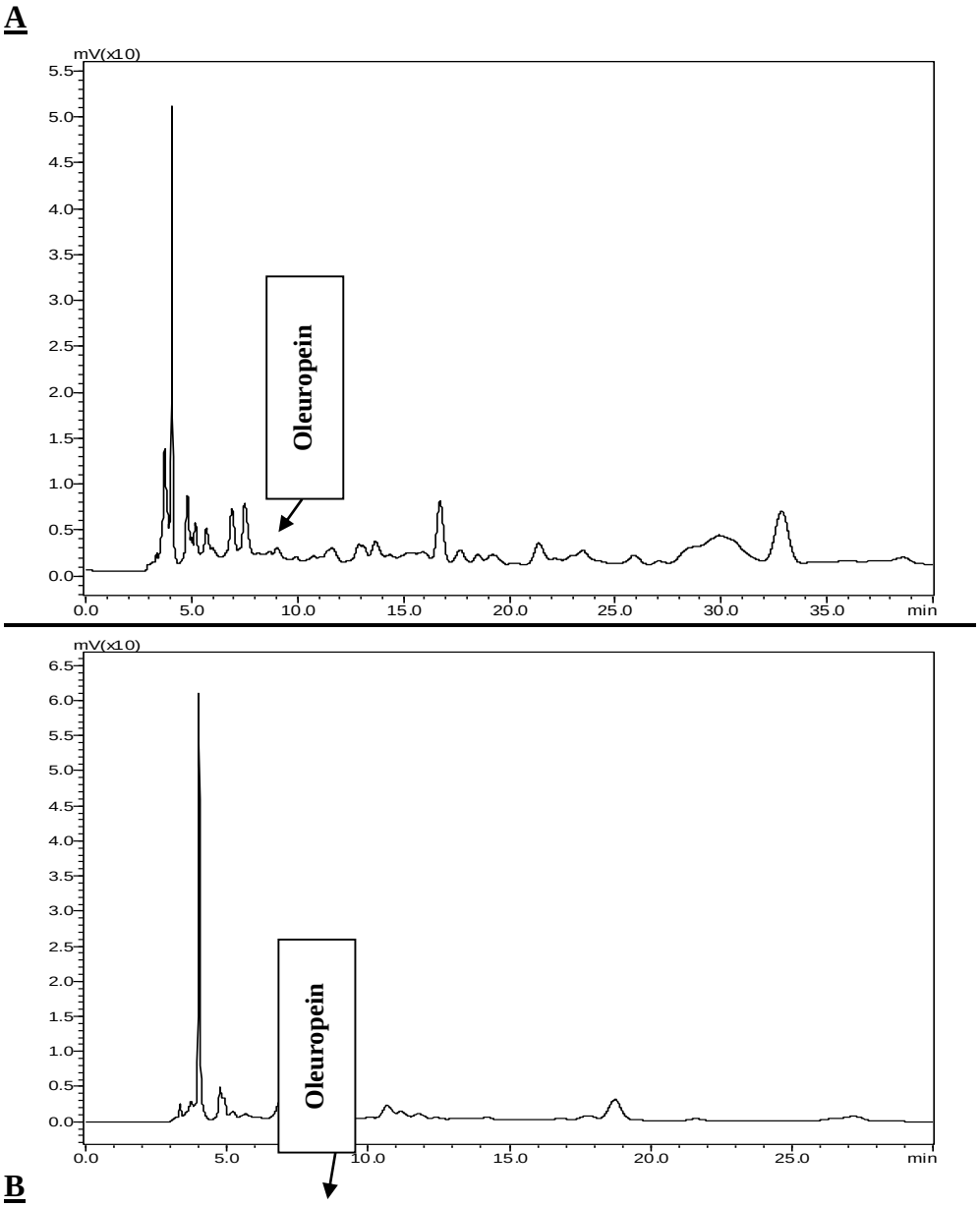

Figure 1. HPLC phenolic profile of olive leaf extract. (A *Dathier*, B *Chemle*l)

Subsequently if we compare our extract with the reference of Oleuropein Standard, we will find that this molecule appear in the run time of 8 minute **(**Figure 2).

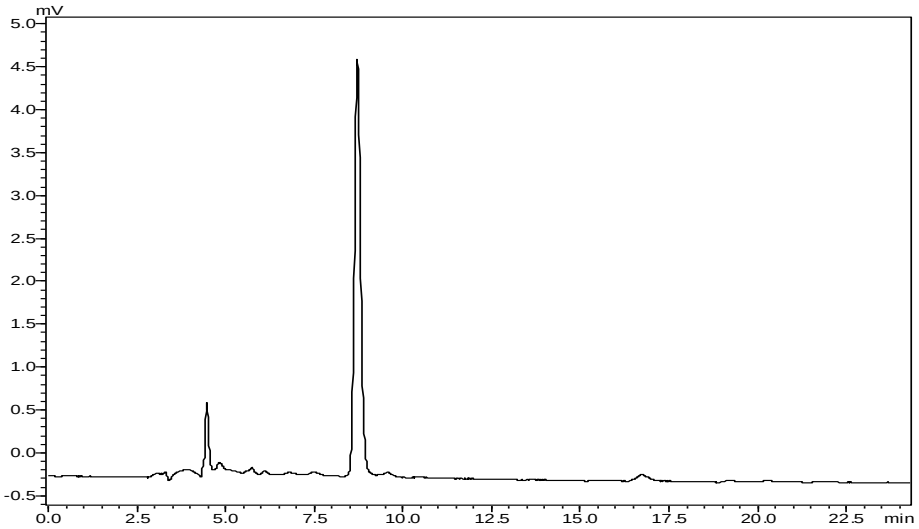

Figure2. HPLC Chromatogram of Oleuropein Standard.

To calculate the Oleuropein amount in the olive leaves extracts, a seven -point calibration curve (R2 = 0.99) was constructed using the standard solutions (Tyrosol) with increasing concentrations (Figure 3).

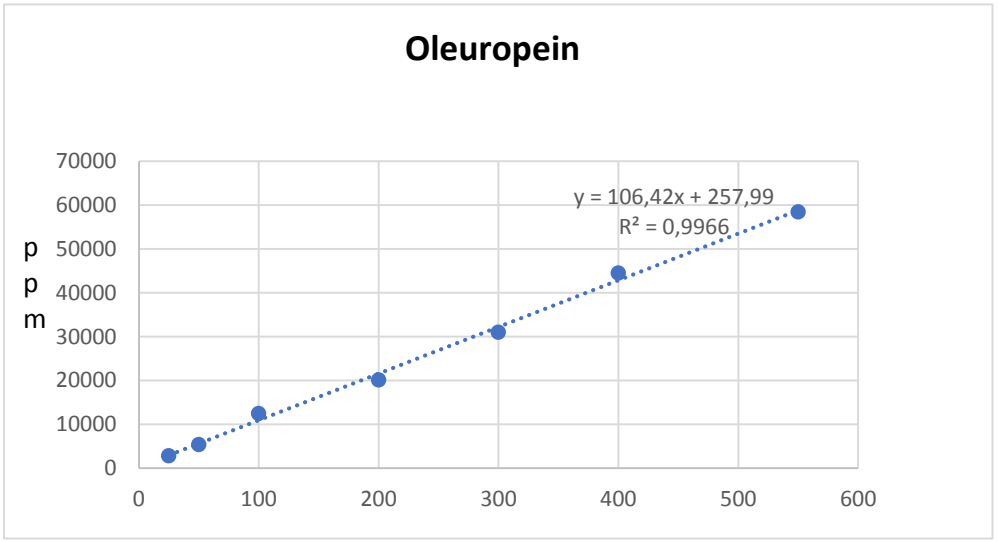

Figure 3. Calibration curve (R^2 = 0.99) of Oleuropein

The olive leaves contain higher amount of polyphenols than olive oil. For example, the amount of Oleuropein, which is the most abundant phenolic compound, ranges from 0.005% and 0.12% in olive oil. On the other hand, in olive leaves, it ranges between 1 and 14% **(Japon-Lujan et *al.*, 2006).** In our study, Oleuropein was present in high amount in extracts from two varieties (Table 2).

Table 2. Quantification of the phenolic compounds in olive leaves extract

	Molecule	Run time min	Quantity mg / g
Chemlel	Oleuropein	7.61	0.703
Dathier	Oleuropein	7,504	0.891

The reference data of Oleuropein content in olive leaves were obtained by the classical technique of HPLC (**Savournin et *al*., 2001; Bouaziz & Sayadi, 2005**). The Oleuropein was eluted at tR = 8 min T (Figure 2). However it was found to be the major phenolic compound (**Japon-Lujan et *al*., 2006; Mourtzinos et *al*., 2007; Altiok et *al*., 2008**). Comparing our results with those of other studies, the concentration of Oleuropein in our station were similar to those recorded in Syrian varieties and Iranian varieties as indicated by **Tayoub et *al*. (2012**). The Oleuropein concentration in leaves of Syrian olive is between 4.3-8.2mg/g, while Iranian olive leaves is between6.1-13-mg/g. (**Afaneh et *al*., 2015**) in their study, mentioned that the amount of Oleuropein in the leaves is 15.6 ± 0.16 mg/g **Nashwa et *al*. (2014**) found a quantity of 540mg/kg, while **Ben Salah et *al*. (2012)** found that it is between 30-52 mg/kg**.**

Antibacterial Activity

The diameter of hibition zone of the methanolic extract of olive leaves (*Chemlal, Dathier*), and two negative and positive controls with four bacterial strains *(E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus).*Were indicated in Table 3 and figure 4.

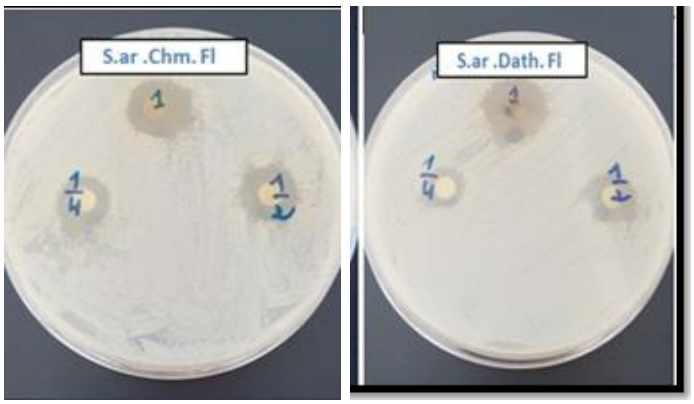

Figure 4. Zone of inhibition of olive leaves extracts against ***Staphylococcus aureus***

Some researchers have sought the total polyphenols content of the olive showing that olive leaves are rich in bioactive phenolic compounds in comparison with olive oil and fruit (**Caponio et *al*., 2001**).

Several studies have shown the biological effects of a wild olive tree that shows its medicinal values. **Bouderba et *al.* (2012**) investigated the antibacterial activity of wild olive leaves in Algeria. However, they performed two different extracts (crude and aqueous) and results in response to the *E. coli* crude extract (15,3 mm), *K .pneumonia* (11,7mm), *P.aerugenosa* (13,3mm), and *S.aureus* (9mm). Furthermore, the aqueous extract of the zone of inhibition is (6,67mm) with *E.coli, K .pneumonia* (19,03mm), *P.aerugenosa* (25,01mm), and *S.aureus* (9,88mm).

Table 3. Zone of inhibition (mm) of olive leaves extracts against four bacterials strains

	Dilution	Escherichia coli	Klebsiella pneumonia)	Pseudomonas aeruginosa,	Staphylococcus aureus)
Chemlel	100%	6.12	7.75	6.87	15.66
	50%	-	7.75	7	13.69
	25%	-	6.62	6.37	12.68
Dathier	100%	6.75	7.75	6.5	17.49
	50%	6.12	10.75	8	12.23
	25%	-	11.62	6.62	8.93
Gentamycine		16.47	16.30	20.60	21.28

Consequently, our extracts have shown an inhibition zone to be less than (**Bouderba et *al.*, 2012**). This difference is a logical view that the oleaster is a plant rich in polyphenols compared to *Olea europeae*. Based on the results of **Aouidi, 2012,** the antimicrobial effect of olive leaves is due to its phenolic composition. Also, it has been reported by **Lee and Lee (2010).** That these researchers also found that a mixture of phenolic compounds of olive leaves has greater antimicrobial activity than those phenolic compounds that were tested individually. They thus demonstrate the presence of antimicrobial effect of phenolic compounds from olive leaves. **Djenane et *al.* (2012**) mentioned that Oleuropein from olive leaves has a very strong vis-à-vis activity of Staphylococcus aureus (inhibition diameter = 30.18 mm).

Conclusion

This work has vividly shown that both methanolic extracts of the *Olea europaea* of two varieties *(Chemlel* and *Dathier)* have antibacterial property against the four potential pathogens. Phenolic compounds are important leaves

constituents because they exhibit antibacterial activity in leaves of *Olea europaea*, Oleuropein was found to be the major phenolic compound

Olive leaves can be included in the list of herbal medicines due to their high antimicrobial potential and lesser side effects. Hence, this extract and their components can be recommended for therapeutic purposes. Also, it can be used as an alternative source of medicine.

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