

## Alpha-amylase inhibitory activity and antioxidant effect of *Olea* var. *europaea sylvestris* leaves extracts.

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### Abstract

Evaluation of medicinal plants for their antidiabetic activities has increased considerably around the world as well as in Algeria. *Olea europaea* var. *sylvestris*, commonly called Ezzeboudj, is traditionally used by Tlemcen population in Algeria, for treating diabetes mellitus. In this work, we studied the antioxidant activity of wild olive leaves crude extracts, by free radical DPPH scavenging method and iron reduction FRAP. On the other hand, evaluation of the ability of the extracts to inhibit  $\alpha$ -amylase activity. The qualitative phytochemical screening carried out on *O. europaea* var. *sylvestris* leaves extracts showed the presence of tannins, sterols and triterpenes, saponins, flavonoids and terpenoids. A quantitative analysis of the crude extracts showed significant levels of total polyphenols and flavonoids in hydroacetic extract, with an amount of  $228,45 \pm 0,03$  mg GAE /g and  $204,8 \pm 0,04$  mg CE /g, respectively. The results obtained from the antioxidant activity showed a high scavenging activity for hydroacetic extract with an  $IC_{50}$  of  $7,95 \pm 0,16$   $\mu$ g/ml. *In vitro* tests carried out on the inhibitory of  $\alpha$ -amylase activity, revealed an inhibitory effects, specifically with hydromethanolic extract in a concentration-dependent manner, with an  $IC_{50}$  value of  $0,91 \pm 0,02$  mg/ml. These findings reveals that *O. europaea* var. *sylvestris* leaves could represent an interesting source of antioxidants and allow to the development of new antidiabetic agents.

**Keywords:** *Olea europaea* var. *sylvestris*, antidiabetic, antioxidant activity,  $\alpha$ -amylase..

### Introduction

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting in defects in insulin secretion, impaired insulin action or both (ADA, 2014). It is one of the most frequent chronic diseases and is the result of the interaction between hereditary and environmental factors (Gu et al, 2010). According to the latest global estimate from the International Diabetes Federation IDF, in 2019, there would be 463 million people with diabetes and, by, 2045, the number would reach 700 million (IDF, 2019).

Type 2 diabetes accounts 90 to 95 % of those with diabetes. This form of diabetes affects patients who have insulin resistance and usually have relative insulin deficiency (ADA, 2014).

Postprandiale blood glucose plays a major role in the onset and development of complications in patient with type 2 diabetes (Chang et al, 2004).

Diabetes mellitus is one of the most common endocrine disorders and medicinal plants continue to play an important role in the management of the disease (Ben jemaa et al, 2017). They constitute a great source of active compounds which can be used to treat many diseases (Gbekley et al, 2015).

Plants and food ingredients with inhibitory effects on digestive enzymes, such like, alpha amylase, and wick affect starch degradation and glucose metabolism, are a potential approach to reduce the increase

in postprandial blood glucose and a subsequent development of diabetes mellitus (Kuritzky et al, 1999).

Algeria is considered one of the richest countries in medicinal plants with over 3164 species (Vasist et Kumar, 2004). *Olea europaea* L. or olive tree, belongs to the Oleaceae family. Olive tree is used in traditional medicine for a wide range of disease in various countries (Hannachi et al, 2013).

Algeria is one of the main Mediterranean countries whose climate is more suitable for the cultivation of these species (Himouret, 2016). Oleaster is widely used by local people in traditional medicine for the treatment of various diseases. The medicinal properties of *Olea europaea sylvestris* trees are mainly attributed to the leaves used as an infusion or decoction (Arab et al, 2013).

In traditional medicines, *Olea europaea* var. *sylvestris* or wild olive leaves are mainly known for their antidiabetic effects (Azzi et al, 2012). Several studies have shown the biological activities of oleaster leaves, including antidiarrhoeal (Zaouani et al 2018) and antitumoral activities (Makowska-Wąs et al, 2017 ; Zerriouh et al, 2017).

Our study is a part of the valorization of *Olea europaea* var. *sylvestris* (Oleaceae family), a medicinal plants used in several regions of the world, including the region of Tlemcen – Algeria. The wild olive constitutes is widely used in traditional remedies in Algeria for its many biological properties, essentially, antidiabetic properties (Azzi et al, 2012).

This study aims to assess the inhibitory effect on the activity of  $\alpha$ -amylase of crude extracts from *O. europaea* var. *sylvestris* leaves. The study was expanded to research and evaluate the antioxidant activity *in vitro*.

## Material and methods

### *Plant material*

The leaves of wild olive (*Olea europaea* var. *sylvestris*), were harvested in January 2019, in the region of El Ourit (Tlemcen city – North west of Algeria).

The leaves were identified and authenticated by us in collaboration with the botanical laboratory of the university of Tlemcen. A voucher specimen was deposited in our laboratory. The plant material was dried in the dark. Once dried, the leaves were ground into fine powder.

### *Preparation of hydromethanolic and hydroacetic extracts*

The powder of wild olive leaves were extracted with 200 ml of the solvent systems : methanol/water (70/30) (v/v) or acetone/water (70/30) (v/v). The extracts were prepared by maceration, with stirring for twenty four hours.

The two extracts were filtered and concentrated under reduced pressure at 60°C using a rotary evaporator, then kept in the dark and stored at 4°C.

The percentage yield of each extracts was calculated as the ratio of the mass of the dry extract to the mass of the ground plant sample.

### *Qualitative phytochemical study*

The main chemical compounds of oleaster were characterized by color reactions and observations under ultraviolet light, using analytical techniques described in literature (Bruneton, 1999 ; Oloyede, 2005).

### *Total polyphenols quantification*

The total phenolic compounds of crude extracts were quantified as follows : 100  $\mu$ l of each extract were mixed with 2 ml of sodium carbonate solution 2 %. After stirring and incubation for five minutes, 100  $\mu$ l of Folin-Ciocalteu reagent 1 N (v/v) were added. The resulting mixtures were

incubated in the dark at room temperature and for 30 minutes. The absorbances were measured at 700 nm against a blank (Vermerris and Nicholson, 2006).

A standard curve is carried out in parallel under the same experimental conditions using gallic acid as positive control in different concentrations.

The results are expressed in milligram gallic acid equivalent per gram of dried plant material (mg EAG/g).

#### *Total flavonoids quantification*

The quantification of flavonoids was carried out by a colorimetric method according to the protocol of Zhishen et al, 1999. A volume (500 µl) of each extract or catechin (positive control) was mixed with 2 ml of distilled water. Then, 150 µl of sodium nitrite solution 15 % were added. After 6 minutes, 150 µl of aluminium chloride  $\text{AlCl}_3$  solution 10 % were added. After 6 minutes of incubation, 2 ml of sodium hydroxide solution 4 % were added. Distilled water was added to obtain a final volume of 5 ml in the different mixtures. After stirring and incubating for 15 minutes, the absorbance was measured at 510 nm against a blank.

Results obtained were expressed in milligrams of catechin equivalent per gram of dried plant material (mg EC/g).

#### *Antioxidant assay*

##### *DPPH free radical scavenging*

A methanolic solution 50 µl of each crude extract at different concentrations was added to 1,95 ml of DPPH solution ( $6 \times 10^{-5} \text{ M}$  in methanol). The studied extracts were tested with methanol as a control, and ascorbic acid was used as a standard antioxidant. The absorbance was determined after 30 minutes at 515 nm (Atoui et al, 2005).

The percent inhibition PI was calculated according to the following formula :

$$PI = \left( \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \right) \times 100$$

##### *Ferric reducing antioxidant power FRAP*

The FRAP method was performed according to the protocol of Yen and Chen, 1995. The different extracts of wild olive leaves and ascorbic acid (positive control) were dissolved in 1 ml of distilled water and mixed with 2,5 ml of 2 M phosphate buffer pH 6,6 and 2,5 ml of ferricyanide potassium  $\text{K}_3\text{Fe}(\text{CN})_6$  1%. The mixtures were incubated at 50°C for 20 minutes. After incubation, 2,5 ml of trichloroacetic acid TCA 10 % were added to stop the reaction. Then, the solutions were centrifuged for 10 minutes and 2,5 ml of supernatants were added to 2,5 ml of distilled water and 0,5 ml of iron chloride solution  $\text{FeCl}_3$  0,1 %. The absorbances were measured at 700 nm against a blank.

##### *Inhibitory effect of *Olea europaea* var. *sylvestris* extracts on $\alpha$ -amylase activity*

Alpha-amylase test was performed according to the 3,5-dinitrosalicylic acid DNSA method adapted from Sigma-Aldrich with some modifications (Berfeld, 1955 ; Oyedemi et al, 2017).

Extracts of wild olive leaves, acarbose, starch solution and alpha amylase (E.C.3.2.1.1 from *Aspergillus oryzae*) were dissolved in phosphate buffer 0,02 M pH 6,9 (2,4 mg/ml of monobasic sodium phosphate and 2,84 of dibasic sodium phosphate) and pre-incubated for 30 minutes at 25°C.

A volume of 200 µl of each extract and acarbose (positive control) at different concentrations were mixed with 200 µl of  $\alpha$ -amylase solution (1,3 U/ml), then incubated for 10 minutes at 25°C. Thereafter, 200 µl of starch solution 1 % were added to each test tube. After stirring and incubating for 10 minutes, 200 µl of DNSA solution (5,3 M of sodium potassium tartrate tetrahydrate dissolved

in 2 M NaOH, then mixed with 96 mM of 3,5-dinitrosalicylic acid solution) were added to stop the reaction.

The different test tubes were immediately placed in a boiling water bath for 10 minutes, then placed in ice-water bath. After dilution of mixtures with 1 ml of ultrapure water, the absorbances were measured against a blank at 540 nm.

This method was carried out in triplicate for each extract as well as for acarbose and a blank for each concentration tested, was prepared by a mixture of 200 µl of phosphate buffer solution, 200 µl of extract/acarbose solution and 200 µl of starch solution. Control tube test contains enzyme and starch solution.

Inhibitory activity was expressed as a percent inhibition and calculated by the following equation :

$$\alpha - \text{amylase inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

### Statistical analysis

All the experiments were carried out in triplicate and expressed in mean  $\pm$  standard error. The IC<sub>50</sub>s (half maximal inhibitory concentration) for each extract were calculated using equations of logarithmic regression plots. And, the EC<sub>50</sub>s (effective concentration of sample whose absorbance equal to 0,5) for each extract were calculated using equations of linear regression plots. The EC<sub>50</sub>s and IC<sub>50</sub>s were calculated using Excel (Office 2016, Microsoft).

## Results

### Extraction yields

Extracts were prepared from the leaves of *O. europaea* var. *sylvestris* by maceration method using two solvent systems : hydromethanolic and hydroacetonc (30/70) (v/v).

The yields observed in Table 1 show that the hydroacetonc extract has a higher yields percent than the hydromethanolic extract.

**Table 1.** Yields of *Olea europaea* var. *sylvestris* extracts

Extracts	Yields (%)	Physical aspect of extracts
Hydromethanolic	12,33	Crystallized dark green
Hydroacetonc	23,41	Crystallized green

### Phytochemical screening, total phenolic and flavonoids quantification

Phytochemical studies carried out on *O. europaea* var. *sylvestris* leaves extracts are reported in table 2.

**Table 2.** Phytochemical screening of *Olea europaea* var. *sylvestris* leaves

Chemical compounds	Hydromethanolic extract	Hydroacetonc extract
Flavonoids	+	+
Tannins	+++	+
Alkaloids	-	-
Coumarins	-	-
Anthraquinones	-	-
Sterols and triterpenes	++	+
Terpenoids	+	+
Saponins	+++	+++
Reducing compounds	-	-

(+) presence ; (-) : absence

We noticed the presence of flavonoids, tannins, sterols, terpenoids and saponins in both crude extracts.

On the other hands, we observed total absence of coumarins, reducing compounds, anthraquinones and alkaloids on oleaster extracts.

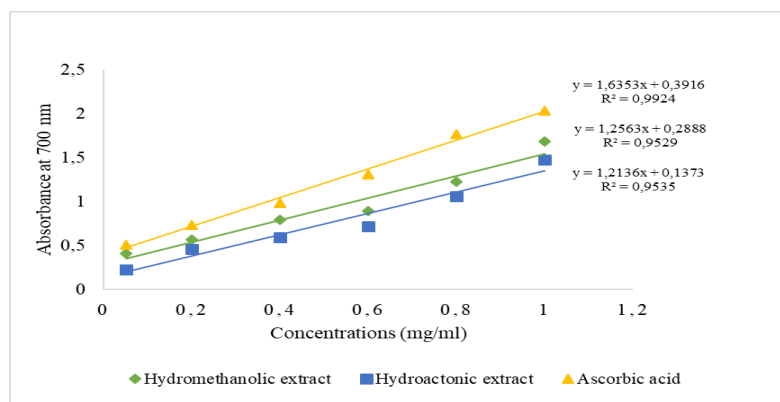
Total polyphenols and flavonoids contents in *O. europaea* var. *sylvestris* extracts were revealed by spectrophotometric assays described above.

The hydroacetic extract has a greater amount of phenolic compounds with a rate of  $228,45 \pm 0,03$  mg GAE /g compared to hydromethanolic extract ( $197,61 \pm 0,02$  mg GAE /g). Similarly, we found that the same extract contains a high content of flavonoids  $204,8 \pm 0,04$  mg CE /g compared to hydromethanolic extract ( $167,7 \pm 0,03$  mg CE /g).

#### Antioxidant activity

Antioxidant activity was evaluated with two methods : iron reduction FRAP and DPPH radical scavenging.

According to the figure 1, hydromethanolic crude extract shows a slightly higher reducing power than the hydroacetic crude extract with absorbances up to 1,68 and 1,47, respectively, at the concentration of 1 mg/ml. We noticed also that the increase in iron reduction is proportional with the increased concentrations of wild olive leaves extracts.



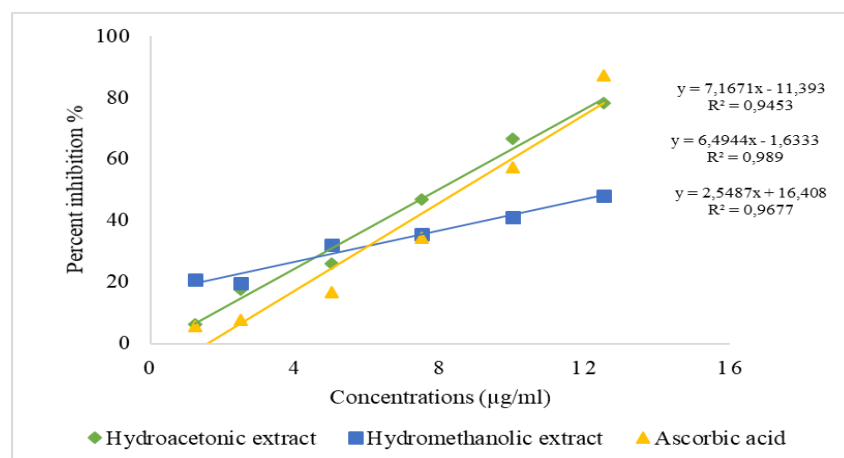
**Figure 1.** Reducing power of crude extracts from *O. europaea* var. *sylvestris* and ascorbic acid

The optical density values obtained were used to calculate the effective concentration  $EC_{50}$  for each extract as well as for ascorbic acid from linear regression curves (table 3). The results reveal that the hydromethanolic extract possess a higher reducing power compared to hydroacetic extract. Their  $EC_{50}$ s are  $0,29 \pm 0,64$  mg/ml and  $0,37 \pm 0,66$  mg/ml, respectively, but relatively lower than the ascorbic acid which is equal to  $0,23 \pm 0,85$  mg/ml.

**Table 3.**  $EC_{50}$  values of crude extracts of *O. europaea* var. *sylvestris* and ascorbic acid

	Hydromethanolic extract	Hydroacetic extract	Ascorbic acid
$EC_{50}$ (mg/ml)	$0,29 \pm 0,64$	$0,37 \pm 0,66$	$0,23 \pm 0,85$

The results of DPPH radical scavenging (figure 2) show that the hydroacetic extract possess high inhibition percentages with low concentrations. Unlike the hydromethanolic extract, which has lower inhibition percentages at the same concentrations.



**Figure 2.** DPPH radical scavenging activity of *O. europaea* var. *sylvestris* leaves extracts

Hydroacetonc extract revealed a highest DPPH radical scavenging activity with  $78,38 \pm 0,16$  % at concentration of  $12,5 \mu\text{g/ml}$ . However, at the same concentration, hydromethanolic extract produced percent of inhibition of  $41,17 \pm 0,05$  %.

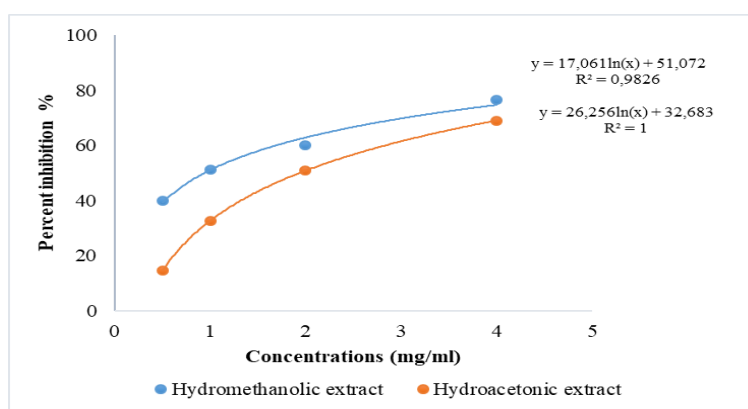
Then, we calculated the  $\text{IC}_{50}$ s for the extracts and ascorbic acid (table 4). Hydroacetonc extract has a lower  $\text{IC}_{50}$  compared to that hydromethanolic extract. Therefore, hydroacetonc extract exhibits better antioxidant activity, and its ability to scavenge the free radical DPPH is higher. However, according to the  $\text{IC}_{50}$ s obtained for both wild olive leaves extracts, ascorbic acid has better DPPH radical scavenging activity.

**Table 4.**  $\text{IC}_{50}$ s values of crude extracts of *O. europaea* var. *sylvestris* and ascorbic acid

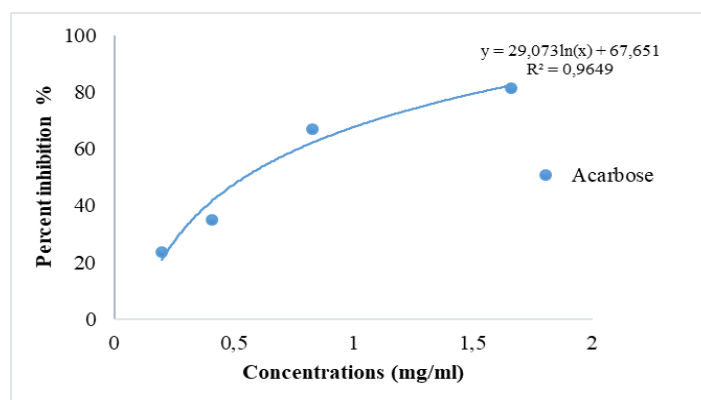
	Hydromethanolic extract	Hydroacetonc extract	Ascorbic acid
$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )	$13,14 \pm 0,18$	$7,95 \pm 0,16$	$3,56 \pm 0,1$

#### Inhibitory effects of *O. europaea* var. *sylvestris* on $\alpha$ -amylase activity

Figure 3 shows percent inhibitions of  $\alpha$ -amylase by hydromethanolic extract, hydroactonic extract and acarbose.



**Figure 3.** Inhibitory effect of *Olea europaea* var. *sylvestris* extracts on  $\alpha$ -amylase activity



**Figure 4.** Inhibitory effect of acarbose on  $\alpha$ -amylase activity

Hydromethanolic extract shows high percent inhibition with low concentrations. At these same concentrations, hydroacetonc extract exhibits lower percent inhibition.

Hydromethanolic extract at 4 mg/ml, showed the highest percent inhibition of 76,55 %. At the same concentration, hydroactonic extract exhibited a lower inhibitory activity with 69,03 % percent inhibition. However, the  $\alpha$ -amylase inhibitory activity exerted by wild olive leaves extracts was not significant as compared with the standard acarbose (1,66 mg/ml with 81,44 % ; figure 4).

Then, we determined the half-maximal inhibitory concentrations  $IC_{50}$ s obtained for *O. europaea* var. *sylvestris* leaves extracts and acarbose (positive control) against  $\alpha$ -amylase. The results showed that hydromethanolic crude extract exerted a higher inhibitory effect on  $\alpha$ -amylase activity than hydroacetonc extract with an  $IC_{50}$ s values of  $0,91 \pm 0,02$  mg/ml and  $1,91 \pm 0,04$  mg/ml, respectively. Meanwhile, both crude extracts of wild olive leaves showed a lower  $\alpha$ -amylase inhibitory activity when compared to acarbose ( $IC_{50} = 0,54 \pm 0,02$  mg/ml).

## Discussion

*Olea europaea* L. and *Olea europaea* var. *sylvestris* are widely distributed in the Mediterranean area, particularly in Algeria. The medicinal properties of these two species are assigned to the leaves, which are known for their beneficial properties for human health, due to their richness in phenolic compounds (Aouidi, 2012 ; Bouarroudj et al, 2016 ; Bouasla et al, 2017).

In the present study, we investigated a possible antidiabetic and antioxidant effects of some crude extracts from the leaves of wild olive trees, traditionally used by the population of Tlemcen – Algeria, to treat some diseases including diabetes mellitus (Azzi et al, 2012).

First, we prepared extracts from the leaves of the plant. The extraction method can affect the quantity and the composition of secondary metabolites of an extract. In addition, several factors can affect the extraction : mode and time of extraction, temperature, nature solvents used, as well as the polarity who allows to solubilize the compounds of similar polarity to the solvent (Green, 2004 ; Ncube et al, 2008).

The leaves of wild olive trees were extracted with 70 % methanol and 70 % acetone, using maceration. The yield obtained for the hydroacetonc extract is higher the hydromethanolic extract.

According to the results obtained from the phytochemical screening carried out on *O. europaea* var. *sylvestris* leaves extracts, we noticed the presence of flavonoids, tannins, sterols, terpenoids and saponins. Tests for coumarins, reducing compounds, anthraquinones and alkaloids were negative on our samples.

Extraction is the main step to extract and isolate phytochemicals from medicinal plants. The efficiency of extraction is affected by chemical nature of molecules in the plant, extraction method chosen and solvent used (Stalikas, 2007).



The quantitative assay carried out on crude extracts of wild olive leaves, aims to determine the content of total polyphenols and flavonoids. The hydroacetic extract has a greater amount of phenolic compounds with a rate of  $228,45 \pm 0,03$  mg GAE /g compared to hydromethanolic extract ( $197,61 \pm 0,02$  mg GAE /g). Similarly, we found that the same extract contains a high content of flavonoids  $204,8 \pm 0,04$  mg CE /g compared to hydromethanolic extract ( $167,7 \pm 0,03$  mg CE /g).

These values are similar to those obtained by Djenane et al, 2019, using methanol 80 % who found a concentration of  $198,7 \pm 3,6$  mg GAE / g of total phenolic compounds in wild olive leaves from Kabylie –Algeria. These authors used methanol 80 % as extraction solvent system.

In addition, the content of total polyphenols and flavonoids in hydromethanolic extract are higher than those obtained for hydroacetic extract. These results indicate that acetone 70 % allows better extraction of phenolic compounds.

Hannachi et al, obtained a total polyphenols and flavonoids contents of Tunisian wild olive leaves using methanol as extraction solvent ranged from  $391,92 \pm 1,41$  to  $831,44 \pm 0,66$  mg GAE / 100 g DW and  $14,02 \pm 2,13$  and  $24,39 \pm 2,41$  mg RE / 100 g DW, respectively (Hannachi et al, 2019).

The variation of the amount of phenolic compounds in olive leaves according to literature depends on climate and agronomic conditions, cultivar, composition of the soil, time of harvesting leaf sample, and age of the trees (Djenane et al, 2019).

According to the results obtained from the qualitative and quantitative phytochemical screening, *Olea europaea* var. *sylvestris* is rich in chemical compounds, represented mainly by oleuropein, a secoiridoid, which is known for its various activities : antidiabetic, antioxidant, antiinflammatory and antitumoral (Khalili et al, 2017 ; Visioli et al, 2002 ; Carnevale et al, 2014 ; Scoditti et al, 2012 ; Han et al, 2009).

The study of Makowska-Was and colleagues, identified flavonoids and phenolic acids in methanol extract of Portugal *O. europaea* var. *sylvestris* harvested in Portugal. The authors identified by HPLC analysis apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, rutin, protocatechuic acid, vanillic acid, gallic acid, p-hydroxybenzoic acid and verbascoside. Oleuropein, a secoiridoids, was identified in the same study by HPLC analysis and the amount was equal to 22,64 mg/g DW (Makowska-Was et al, 2017).

Furthermore, Mechchate and colleagues have analyzed oleaster leaf flavonoids by HPLC with triple quadrupole mass spectrometry. The authors have identified five flavonoids (amentoflavone, quercetin-3-*O*-glucoside, quercetin-3-*O*-hexose-deoxyhexose, luteolin-7-*O*-glucoside and rutin. Another molecule belonging to secoiridoids was identified, oleuropein, generally found in oleaceae family (Mechchate et al, 2020).

Therefore, we were interested in this study, in evaluating the antioxidant effect of crude extracts from the leaves of *O. europaea* var. *sylvestris*.

Antioxidant activity was assessed by the iron reduction method FRAP, which represent a significant indicator for the antioxidant power of plants (Mezouar et al, 2014). FRAP is a method based on an electron transfer reaction which measures the reduction of ferric ion ( $\text{Fe}^{3+}$ ) - ligand complex to a blue colored ferrous complex ( $\text{Fe}^{2+}$ ) by antioxidants (Antolovich et al, 2002).

The results revealed that the reducing capacity of the hydromethanolic crude extract is slightly higher than the hydroacetic crude extract with absorbances up to 1,68 and 1,47, respectively, at the concentration of 1 mg/ml. We noticed also that the increase in iron reduction is proportional with the increased concentrations of wild olive leaves extracts.

The results effective concentration  $\text{EC}_{50}$  for oleaster extracts, reveal that the hydromethanolic extract possess a higher reducing power compared to hydroacetic extract. Their  $\text{EC}_{50}$ s are  $0,29 \pm 0,64$  mg/ml and  $0,37 \pm 0,66$  mg/ml, respectively, but relatively lower than the ascorbic acid which is equal to  $0,23 \pm 0,85$  mg/ml.



The ability of the extracts of *O. europaea* var. *sylvestris* to give hydrogen was evaluated using a DPPH free radical : the amount required to reduce the initial concentration of DPPH by 50 % is a parameter used widely for measuring antioxidant activity (Brand-William et al, 1995).

DPPH is a method based on a hydrogen atom transfer reaction. In the initial form, DPPH has a dark purple color, which turns in yellow in his reduced form (Romanet et al, 2019).

The results of DPPH radical scavenging show that the hydroacetonc extract gave high inhibition percentages with low concentrations. Unlike the hydromethanolic extract, which has lower inhibition percentages at the same concentrations.

Hydroacetonc extract showed a highest DPPH radical scavenging activity with  $78,38 \pm 0,16$  % at concentration of  $12,5 \mu\text{g/ml}$ . However, at the same concentration, hydromethanolic extract produced percent of inhibition of  $41,17 \pm 0,05$  %.

The  $\text{IC}_{50}$  is the concentration of antioxidant required to reduce 50 % of the initial concentration of the free radical DPPH (Deng et al, 2011 ; Granados-Guzmán et al, 2017).

Hydroacetonc extract has a lower  $\text{IC}_{50}$  compared to that hydromethanolic extract. Therefore, hydroacetonc extract exhibits better antioxidant activity, and its ability to scavenge the free radical DPPH is higher.

A study was carried out by Djenane and colleagues on the antioxidant activity of macerated hydromethanolic extract (20 : 80 / v : v) from the leaves of *Olea europaea* var. *sylvestris* from Borj Ménaïel – Boumerdès (Kabylie region – Algeria).

The authors obtained similar results for the scavenging method of free radical DPPH and the  $\text{IC}_{50}$  value was  $19,2 \pm 1,6 \mu\text{g/ml}$ . In addition, the authors determined the antioxidant activity index which is equal to  $4,2 \pm 0,1 \mu\text{g/ml}$ , which represents a very strong activity of the wild olive leaves extract (Djenane et al, 2019).

Hannachi and colleagues, evaluated the antioxidant power of hydromethanolic extract of cultivated (*Olea europaea* var. *europaea*) and wild olive (*Olea europaea* var. *sylvestris*) leaves from two regions in Tunisia. The results showed a significant DPPH radical scavenging activity with values from  $3,54 \pm 0,06$  to  $13,11 \pm 0,16$  TEAC mM (mM Trolox equivalent antioxidant capacity) for wild olive leaves extract and a similar scavenging activity for cultivated olive leaves with values from  $7,09 \pm 0,12$  to  $13,62 \pm 0,17$  TEAC mM (Hannachi et al, 2019).

The antioxidant activity of our crude extracts can be explained by the presence of total polyphenols and flavonoids. Other studies suggests a strong correlation between antioxidant activity and high polyphenols content (Pilluzza and Bullitta, 2011 ; Lahmadi et al, 2019), which means that polyphenols are the main chemical compounds which contribute significantly to the antioxidant activity of medicinal plants (Ramos-Escudero et al, 2015 ; Pilluzza and Bullitta, 2011).

This is in agreement with the findings obtained by Djenane and colleagues, who report that wild olive leaves are characterized by a high content of oleuropein, and a lower quantity of hydroxytyrosol (Djenane et al, 2019). Indeed, some authors have shown that oleuropein have scavenge ABTS radicals in trolox equivalent antioxidant activity test (Benavente-Garcia et al, 2000 ; Jemai et al, 2009 ; Ivanov et al, 2018).

The results obtained from the phytochemical study and antioxidant activity of *Olea europaea* var. *sylvestris* arouse interest in studying other potential biological activities, whose antidiabetic activity.

One of the effective strategies for the management of type 2 diabetes is the inhibition of hydrolysis of complex polysaccharides by pancreatic  $\alpha$ -amylase and glucose uptake limitation by inhibiting intestinal  $\alpha$ -glucosidase enzyme (Stojkovic et al, 2019). The potential role of herbal remedies as inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase has been reviewed by several authors (Ye et al, 2010 ; Sales et al, 2012 ; Governa et al, 2018 ; Abu-Odeh and Talib, 2021).

In the present study, we evaluated *in vitro*, the potential inhibitory effect of *Olea europaea* var. *sylvestris* leaves extracts on *Aspergillus oryzae*  $\alpha$ -amylase activity. Hydromethanolic crude extract exhibited a greater inhibitory effect on  $\alpha$ -amylase activity than hydroacetic extract with an  $IC_{50}$  values of  $0,91 \pm 0,02$  mg/ml and  $1,91 \pm 0,04$  mg/ml, respectively. Meanwhile, both crude extracts of wild olive leaves showed a lower  $\alpha$ -amylase inhibitory activities when compared to acarbose ( $IC_{50} = 0,54 \pm 0,02$  mg/ml).

The results obtained above suggest that *O. europaea* var. *sylvestris* leaves extracts could act in the digestive tract by inhibiting the digestive  $\alpha$ -amylase activity, and consequently, decreasing postprandial hyperglycemia (Komaki et al, 2003 ; Hadrich et al, 2015).

The antidiabetic effects observed are in agreement with the results obtained from some studies of mechanisms of action of oleuropein and hydroxytyrosol isolated from olive leaves. In fact, these two compounds affect carbohydrate metabolism by inhibiting intestinal maltase, human sucrase and glucose transport across Caco-2 cell monolayers and glucose uptake by GLUT 2 in *Xenopus oocytes* (Kerimi et al, 2019).

Furthermore, in another study of Hadrich and colleagues, hydroxytyrosol isolated from olive tree leaves and oleuropein showed an inhibitory effects on  $\alpha$ -amylase and  $\alpha$ -glucosidase activities (Hadrich et al, 2015). In addition, Mechchate and colleagues evaluated the antidiabetic activity of flavonoids from oleaster leaves. The authors show that flavonoids exhibit an antihyperglycemic effects in alloxan-induced diabetic mice during 28 days of treatment. In the same study, the mode of action of four flavonoids (amentoflavone, quercetin, rutin and luteolin-7-O-glucoside) and oleuropein from oleaster leaves against receptors, including  $\alpha$ -amylase, which have a high impact on the management of diabetes and its complications has also been studied using molecular docking. The in-silico mode of action of these compounds revealed inhibition of  $\alpha$ -amylase showing their affinity for the active site. The highest affinity was noted for amentoflavone and oleuropein has the lowest affinity compared to other compounds tested (Mechchate et al, 2020).

## Conclusion

The results of this study suggest the multiple effects of *Olea europaea* var. *sylvestris* crude extracts. Total polyphenols and flavonoids contents determined could be responsible of these activities, which was reflected in the antioxidant capacity of its extracts as well as their inhibitory effects on alpha amylase activity.

These results confirm the traditional uses of *O. europaea* var. *sylvestris* leaves in the treatment of diabetes mellitus in Tlemcen region – Algeria. However, further studies are needed to elucidate the composition of phenolic bioactive compounds and determine their molecular mechanisms of action, which could represent a promising source as new drugs.

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