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Research Article

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Phytochemical analysis and alpha-amylase inhibitory property of olive (*Olea europaea* L.) leaves extracts

Dounia MEZOUAR*, Mohammed AISSAOUI, Amina BENMESSAOUD, Farid Boucif LAHFA

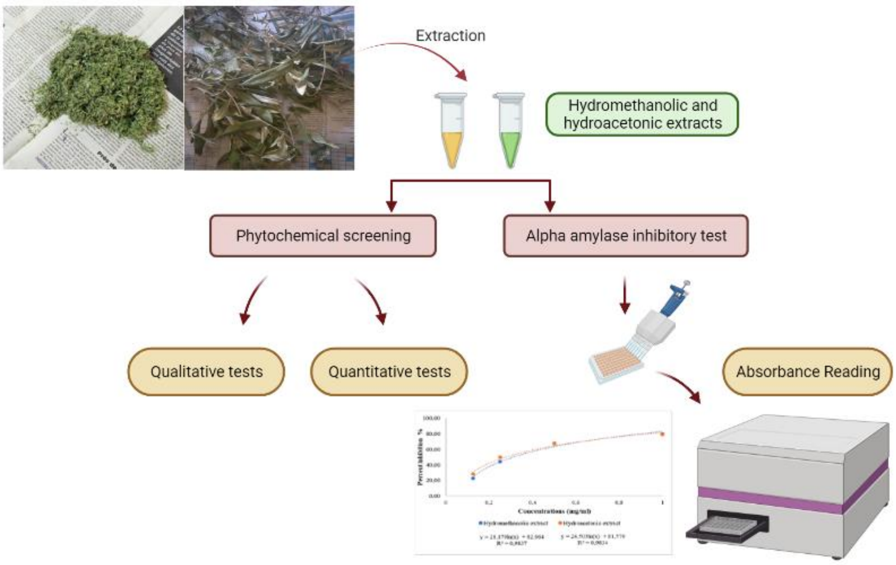
Laboratory Antibiotics, Antifungal : Physico-Chemical, Synthesis and Biological Activity, Faculty SNV-STU, Abou Bekr Belkaïd University, BP 119, Tlemcen, Algeria

*Corresponding author email : dounia.mezouar@univ-tlemcen.dz ; Tel: (+213) 553-27-43-58

Highlights

- Hydroacetonic and hydromethanolic crude leaves extracts from cultivated olive tree are rich in phenolic compounds including flavonoids ;
- These crude extracts exhibit an antidiabetic activity with an inhibitory effect against alpha amylase activity ;
- Olive tree leaves could help to improve the level of postprandial hyperglycemia, and can be a source of new antidiabetic molecules.

Graphical abstract



Abstract

Evaluation of medicinal plants for their antidiabetic activities has increased considerably around the world as well as in Algeria. *Olea europaea*, commonly called Zeytoune, is traditionally used by Tlemcen population in Algeria, for treating diabetes mellitus.

In this work, we evaluate the capacity of *O. europaea* leaves crude extracts to inhibit *in vitro* α-amylase activity.

The qualitative phytochemical screening carried out on *O. europaea* 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 hydroacetonic extract, with an amount of 802.57 ± 0.001 mg GAE/ g and 359 ± 0.002 mg CE/ g, respectively.

In vitro tests carried out on the inhibitory of α-amylase activity, revealed an inhibitory effects, specifically with hydroacetonic extract in a concentration-dependent manner, with an IC_{50} value of 0.27 ± 0.02 mg/mL.

These findings reveals that *O. europaea* leaves extracts could represent an interesting source of bioactive compounds and allow to the development of new antidiabetic agents.

Keywords: *Olea europaea*; phytochemical screening; antidiabetic; α-amylase.

1. Introduction

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting in defects in insulin secretion, impaired insulin action or both (Mohan & Dutta, 2022). 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; Frank et al., 2022). 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 ; Liyanagamage et al., 2020). They constitute a great source of active compounds which can be used to treat many diseases (Gbekley et al., 2015 ; Choudhury et al., 2018).

Plants and food ingredients with inhibitory effects on digestive enzymes, such like, alpha amylase, and wich 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 contries in medicinal plants with over 3164 species (Zatout et al., 2021). *O. 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 contries (Hannachi et al., 2013).

Algeria is one of the main Mediterranean contries whose climate is more suitable for the cultivation of these species (Himour, 2016). *O. europaea* is characterized by its richness in active compounds such as polyphenols and flavonoids wich have an important and different properties. The main classes of phenols in olive tree are phenolic acids, phenolic alcohols, flavonoids and secoiridoids (Silva et al., 2006).

In traditional medicines, *O. europaea* or cultivated olive leaves are, mainly known for their antidiabetic effects (Azzi et al., 2012). Several studies have shown the biological activities of Olive leaves, including hypotensive (Lockyer et al., 2017), antidiabetic (De Bock et al., 2013 ;

Al-Attar et Alsalmi, 2019), hypocholesterolaemic (Cheurfa et al., 2019) and antitumoral activities (Barrajón-Catalán et al., 2015 ; Boss et al., 2016).
Our study take part of the valorization of *O. europaea* (Oleacea family), a medicinal plants used in several regions of the world, including the region of Tlemcen – Algeria. Olive is widely used in traditional remedies in Algeria for its many biological properties, essentially, antidiabetic properties (Azzi et al., 2012 ; Hamza et al., 2019).
This study aims is to assess the inhibitory effect on the activity of α-amylase of crude extracts from cultivated *O. europaea* leaves.

2. Material and methods

2.1. Plant material

Leaves of cultivated olive (*O. europaea* L.), were harvested in January 2019 and 2020, 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.

2.2. Preparation of hydromethanolic and hydroacetonic extracts

The powder of olive leaves was 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 decoction, with stirring for three hours. The two extracts were filtered and concentrated under reduced pressure at 60°C using rotary evaporator, then kept in the dark and stored at 4°C. The percentage yield of each extract was calculated as the ratio of the mass of the dry extract to the mass of the ground plant sample.

2.3. Phytochemical study

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

2.4. Total polyphenols quantent

The total phenolic compounds of crude extracts were quantified as follows: 100 µL of each extract were mixed with 2 mL of sodium carbonate solution 2 %. After stirring and incubation for five minutes, 100 µL 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 aquivalent per gram of dried plant material (mg EAG/g).

2.5. Total flavonoids quantification

The quatification 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 (positif 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 $AlCl_3$ 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).

2.6. Inhibitory effect of *Olea europaea* extracts on α-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 *O. europaea* leaves, acarbose, starch solution and alpha amylase (E.C.3.2.1.1 from *Aspergillus orizae*) 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 α-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. A blank for each concentration tested, was prepared by mixing 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 :

$$\alpha - \text{amylase inhibition} = \left(\text{Acontrol} - \text{Asample} / \text{Acontrol} \right) \times 100$$

2.7. Statistical analysis

All the experiments were carried out in triplicate and expressed in mean ± standard error. The IC_{50}s (half maximal inhibitory concentration) for each extract were calculated using equations of logarithmic regression plots. The IC_{50}s were calculated using Excel (Office 2016, Microsoft).

3. Results and 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 and Bouasla, 2017).
In the present study, we investigated a possible antidiabetic effect of some crude extracts from leaves of cultivated olive trees (*O. europaea*), 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 study of medicinal plants begins with the extraction methods, which are an essential step to extract the bioactive constituents of plant materials (Azwanida, 2015). The extraction method can affect the quantity and the composition of secondary metabolites of an extract. In addition, several factors can affect the extraction : method and time of extraction, temperature, nature of 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 olive trees were extracted with 70 % methanol and 70 % acetone, using decoction method. The yield obtained for the hydroacetonic extract is higher the hydromethanolic extract (Table 1).

Table 1. Yields of *Olea europaea* extracts.

Extracts	Rendements (%)	Physical aspect of extracts	Solubility
Hydromethanolic	23.05	Crystallized yellow	Distilled water
Acetonic	30	Crystallized green	Distilled water

Qualitative phytochemical screening is a simple, fast and inexpensive method who provides us a quick answer on the different types pf phytochemical molecules in an extract and is an important method in the phytochemical study of bioactive compounds (Sasidharan et al., 2011).

In our study, phytochemical studies carried out on *O. europaea* leaves extracts are reported in table 2. According to the results obtained, we noticed the presence of flavonoids, tannins, sterols and triterpens, terpenoids and saponins. Tests for coumarins, reducing compounds, anthraquinones and alkaloids were negative on our samples.

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

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

(+) presence ; (-) : absence

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 quatitative assay carried out on crude extracts of *Olea europaea* leaves, aims to determine the content of total polyphenols and flavonoids. The hydroacetonic extract has a greater amount of phenolic compounds with a rate of 802.57 ± 0.001 mg GAE /g compared to hydromethanolic extract (720.11 ± 0.003 mg GAE /g). Similarly, we found that the same extract contains a high content of flavonoids 359 ± 0.002 mg CE /g compared to hydromethanolic extract (329 ± 0.001 mg CE /g).

These values are similar to those obtained by Zaïri et al. (2020), who founds a concentration ranging from 480.34 ± 1.36 to 546.06 mg GAE / g of total phenolic compounds and 506.4 ± 1.96 to 605.25 ± 3.17 of flavonoids in olive leaves from Tunisian Meski and Chemlali regions, respectively. These authors used distilled water as extraction solvent system to prepare infusion extracts (Zaïri et al., 2020). In addition, the content of total polyphenols and flavonoids in hydroacetonic extract are higher than those obtained for hydromethanolic extract. These results indicate that acetone 70 % allows better extraction of phenolic compounds.

Hannachi et al, (2019) obtained a total polyphenols and flavonoids contents of two Tunisian olive cultivar leaves (Zarrazi and Chemlali region), using methanol as extraction solvent ranged from 750.69 ± 0.66 to 579.00 ± 0.66 mg GAE / 100 g DW and 17.62 ± 2.09 and 22.95 ± 0.77 mg RE / 100 g DW, respectively (Hannachi et al., 2019). The variation of phenolic compounds amounts in olive leaves according to litterature 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* 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). In the study of Dekdouk et al, (2015), analysis by RP-HPLC method coupled to Diode-Array, identified major phenolic coupounds in ethyl acetate extract of some selected *O. europaea* fruit cultivars harvested in Italy and Algeria. The authors identified fourteen coupounds divided into four classes: phenolic acids (p-hydroxybenzoic acid, vanillic acid, caffeic acid, gallic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid), flavonoids (luteolin and chrysoeriol), phenolic alcohols (hydroxytyrosol and tyrosol) and secoiridoids (oleuropein and verbascoside) (Dekdouk et al., 2015). According to Kiritsakis et al, (2010), oleuropein is the main phenolic compound of olive leaves, representing 9 % of total leaves weight (dry matter) (Kiritsakis et al., 2010). The results obtained from the phytochemical study of *O. europaea* arouse interest to study 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 α-amylase and glucose uptake limitation by inhibiting intestinal α-glucosidase enzyme (Stojkovic et al., 2019). The potential role of herbal remedies as inhibitors of α-amylase and α-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* leaves extracts on *Aspergillus oryzae* α-amylase activity. In Figure 1, hydromethanolic extract shows high percent inhibition with low concentrations. At the same concentrations, hydroacetonic extract exhibits lower percent inhibition, and consequently, a slightly better activity. Hydromethanolic extract at 1 mg/mL, showed the highest percent inhibition of 79.91 %. At the same concentration, hydroactonic extract exhibited similar inhibitory activity with 79.05 % percent inhibition.

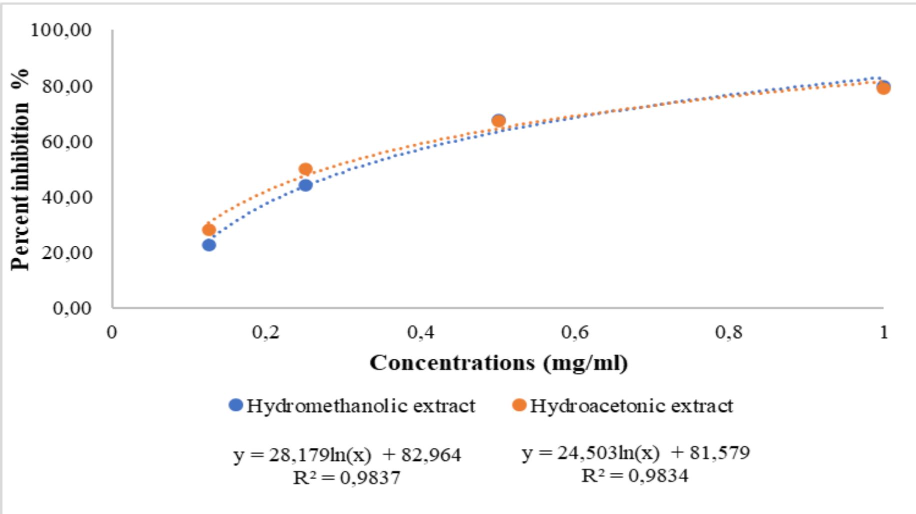


Figure 1. Inhibitory effect of *Olea europaea* crude extracts on α-amylase activity.

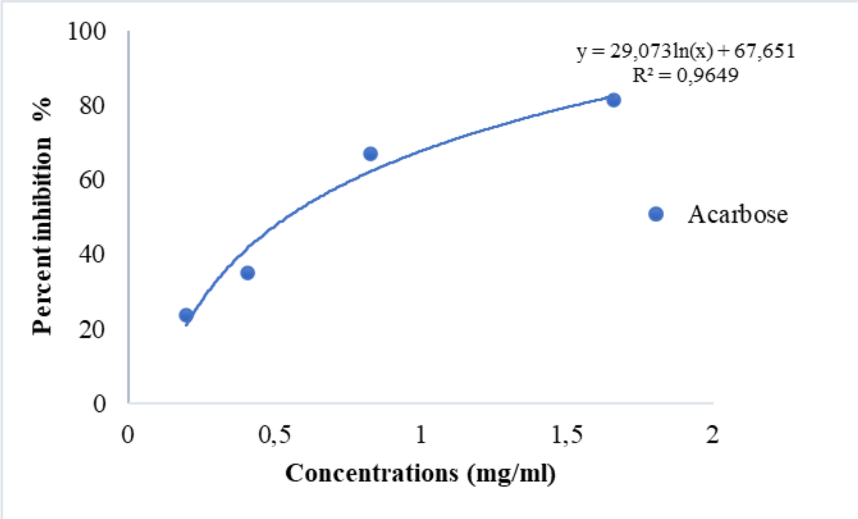


Figure 2. Inhibitory effect of acarbose on α-amylase activity.

The α-amylase inhibitory activity by cultivate olive leaves extracts was not significant as compared with the standard acarbose (1.66 mg/mL with 81.44 % ; Figure 2), but the results showed that the crude extracts contained bioactive molecules that can inhibit the enzyme activity.

Then, we determined the half-maximal inhibitory concentrations IC_{50}s obtained for *O. europaea* leaves extracts and acarbose (positive control) against α-amylase. Hydroacetonic crude extract exhibited a similar inhibitory effect on α-amylase activity as the hydromethanolic extract with an IC_{50}s values of 0.27 ± 0.02 mg/mL and 0.31 ± 0.03 mg/mL, respectively. Meanwhile, both crude extracts of olive leaves showed a slightly lower α-amylase inhibitory activities when compared to acarbose (IC_{50} = 0.54 ± 0.02 mg/mL).

The results obtained above suggest that *O. europaea* leaves extracts could act on the digestive tract by inhibiting the digestive α-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 actions 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 et al. (2015), hydroxytyrosol isolated from olive tree leaves and oleuropein showed an inhibitory effect on α-amylase and α-glucosidase activities (Hadrich et al., 2015). In addition, Guex et al. (2019) evaluated the antidiabetic activity of ethanolic extract from olive leaves. The authors show that ethanolic extract exhibit an improvement effects on glucose levels, inflammatory and metabolic markers in streptozotocin-induced diabetic rats compared to diabetic control rats (Guex et al., 2019). In another study, pure oleuropein was extracted from olive leaves and used in the treatment of induced type 1 alloxan-diabetic rats. Oleuropein showed a significant decreasing in glucose levels and elevation of in vivo antioxidant reduced glutathione GSH (Qadir et al., 2016).

4. Conclusion

The results of this study suggest the multiple effects of *O. europaea* crude extracts. Total polyphenol and flavonoid contents determined could be responsible of these activities, which were reflected on the inhibitory effects of crude extracts on alpha amylase activity. These results confirm the traditional uses of *O. europaea* 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 sources of new drugs.

Conflict of interest

The athors declare that there are no conflicts of interest.

Author Contribution Statement

Dounia MEZOUAR : proposed the experimental protocols, carried out the experiments of this study and wrote the manuscript ; Mohammed AISSAOUI contributed in the realization of certain experiments ; Amina BENMESSAOUD : carried out the experiments ; Farid Boucif LAHFA : proposed this study.

ORCID

Dounia MEZOUAR : 0000-0003-4944-0237

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