Original Research Article

Pistacia lentiscus L. fatty oil and its unsaponifiable matter: Inhibitory potential against key enzymes involved in Alzheimer's disease and type II diabetes

Abstract

The inhibition of key enzymes involved in type II diabetes and Alzheimer's disease is an effective therapeutic approach to the management of these health disorders.

In the present study, *Pistacia lentiscus* L. fatty oil (PLFO) was investigated against α-amylase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase activities to shed light on its therapeutic potential in controlling type II diabetes and Alzheimer's disease. Similarly, the unsaponifiable matter (USM) of this oil was isolated to be screened for the same activities. The antioxidant power of both extracts was assessed as well using DPPH, ABTS, and CUPRAC assays. The results revealed that PLFO had a low antioxidant effect mainly due to its USM. The potent antidiabetic effect of PLFO was also attributed to its USM whereas the whole oil was responsible for the neuroprotective effect. These findings suggest that PLFO and its USM could be used as a source of natural compounds for the management of type II diabetes and Alzheimer's disease.

Keywords: *Pistacia lentiscus L. fatty oil; unsaponifiable matter; α-amylase; α-glucosidase; acetylcholinesterase; butyrylcholinesterase.*

1. INTRODUCTION

Despite the wide emergence of innovation trends in the pharmaceutical fields and medication, many populations around the globe are still relying on traditional medicine to meet their health care needs. In Algeria, as in many other African countries, traditional medicine can be considered a primary option in treating many health conditions. Starting from its cultural and natural heritage, the traditional-medicine-oriented community believes that the use of *Pistacia lentiscus* L. has always been an effective approach in dealing with common diseases.

This plant locally named "Derou" is one among the most common shrubs in the Mediterranean areas and gives a fatty oil usually used in the treatment of wounds and burns. It is characterized by a large morphological and chemical variability and an important genotypic polymorphism [1,2].

The most famous variety is without any doubt the mastic tree occurring on Chios island in Greece which provides the appreciated mastic of Chios (also called Chios Gum Mastic or mastiha) well known all over the world for its multiple biological activities and applications [3].

The variety that thrives in Algeria provides a kind of resin, rather pasty or liquid, that does not harden in mastic [4]. However, this variety is mainly used for the extraction of valuable fatty oil that has a long history in the folk medicine of many regions in the country. Using traditional tools, the oil is pressed from the black ripe fruits of *Pistacia lentiscus* L. and is used as a traditional remedy to soothe gastralgia and diarrhea but also as a food oil. The oil is also locally applied for wounds scabies and rheumatism [5,6].

Recent studies have revealed that PLFO is essentially composed of fatty acids with a predominance of monounsaturated fatty acids (52%). Polyunsaturated fatty acids constitute about 18% of the oil and are represented mainly by linoleic acid. The oil contains also sterols, tocopherols, carotenoids, and chlorophyll [7-

10]. This oil is also considered as a good source of phenolic compounds with almost 40 compounds including Gallic acid, Tyrosol, Vanillic Acid, Kaempferol, etc. [7,11].

The biological activities of PLFO were as well highlighted by many authors. This oil was reported to have wounds and burn healing effect [12-15]. This latter was attributed to the unsaponifiable matter isolated from the oil [16]. In addition, the oil has antioxidant activity [7,17,18], antibacterial [19-21], and antifungal activities [20]. Many other properties have been recognized for this oil such as anti-inflammatory effect [22,23], antiasthmatic [23], antiproliferative [24], anti-hypercholesterolemic [25], as well as a gastroprotective and antiulcerogenic effects [26].

The oil prevents also learning and memory disorders induced by lipopolysaccharide and attenuates oxidative damage in brain tissue and liver in rats, by antioxidant, neuroprotective, and acetylcholinesterase inhibitor effects [27].

Nevertheless, there are no previous studies reporting the *in vitro* antidiabetic and neuroprotective effects of *P. lentiscus* L. fatty oil. Furthermore, the unsaponifiable matter has not received enough attention from authors [16]. Thus, the present work is considered the first attempt to assess the *in vitro* antidiabetic and neuroprotective activities of *Pistacia lentiscus* L. fatty oil and its unsaponifiable matter.

2. MATERIALS AND METHODS

2.1. Plant material

Black mature fruits of *Pistacia lentiscus* L. were harvested from Mount "Houara" located in Guelma (Northeastern Algeria), in October 2018. The fruits were washed and dried in a shade and ventilated place.

Figure 1. Fruits of *Pistacia lentiscus* L. (Guelma 2018)

2.2. Extraction of *Pistacia lentiscus* L. fatty oil (PLFO)

1kg of dried fruits of *P. lentiscus* L. were pressed at room temperature (20°C) by a cold-pressing machine (XING FA). Fruit oil was then stored at 4°C until use.

2.3. Separation of unsaponifiable matter (USM)

The saponification of PLFO was performed according to the method of the European Pharmacopoeia, 7th edition, modified according to Boulebda [28,16].

50 g of PLFO were heated with 200 mL of an alcoholic potassium hydroxide solution (KOH 2N) in a flask fitted with a reflux condenser until total saponification (1 h). After cooling, the ethanol was evaporated under vacuum using a rotary evaporator. Then, 100 mL of distilled water was added to the residue and transferred to a separatory funnel, where the liquid was shaken carefully with three quantities, each of 150 mL of diethyl ether.

Ether layers were combined and dried over anhydrous sodium sulfate (Na_2SO_4). Ether was finally removed to recover the unsaponifiable fraction that was stored at 4°C until use.

2.4. Sample preparation and spectral measurements

Before each assay, stock solutions of each extract were prepared. 4 mg of PLFO and 4 mg of USM were dissolved in 1 mL of methanol and shaken in a vortex. Each stock solution was diluted to give seven concentrations. For the determination of Total Polyphenolic Content (TPC) and Total Flavonoid Content (TFC), the concentration of stock solutions was 1 mg/mL. All assays were performed in a 96-well microplate, and the absorbances were measured by the microplate reader (Enspire Multimode plate reader, Perkin Elmer). For each independent experiment, all samples were tested in triplicate.

2.5. Determination of Total Polyphenolic Content (TPC)

The TPC was evaluated using the Folin-Ciocalteu reagent, according to a microplate assay method described by Müller et al. [29].

Twenty microliters of each aqueous extract (PLFO and USM as 1 mg/mL) were mixed with 100 mL of 1:10 diluted Folin-Ciocalteu reagent and 75 mL of sodium carbonate solution (7,5%) in a 96-well microplate. After 2 h in darkness at room temperature, the absorbance was measured at 740 nm in the microplate (Perkin Elmer Enspire, Singapore). Gallic acid was used as a standard for calibration and construction of a linear regression line and water was blank. The TPC was calculated as Gallic acid equivalents (GAE) in µg/mg of extract.

2.6. Determination of Total Flavonoid Content (TFC)

For the determination of TFC, the method described by Topçu et al. [30] was used with slight modifications. 50 µL of each sample were added to 10 µL of 10% aluminum nitrate, 10 µL of 1M potassium acetate, and 130 µL of methanol. After 40 minutes of incubation at room temperature, the absorbance was evaluated at 415 nm in the microplate reader. Quercetin was used as a standard, and TFC was expressed as quercetin equivalent (QE) in µg/mg of extract.

2.7. Assessment of antioxidant activity

2.7.1. DPPH free radical scavenging activity

The inhibitory activity of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is determined spectrophotometrically by the DPPH assay [31]. α-tocopherol, BHT, and BHA were used as antioxidant standards. Thus, to 40 µL of sample solution at different concentrations, we added 160 µL freshly prepared DPPH methanol solution. After 30 min at room temperature in dark, the decrease in absorption of the DPPH solution was measured at 517 nm. Methanol was used as a control. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The results were expressed in terms of IC_{50} (The concentration of extract required to inhibit 50% of DPPH radical concentration).

2.7.2. ABTS radical cation scavenging activity

The scavenging activity against the ABTS (2,2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid)) cation radical was measured according to the slightly modified method of Re et al. [32].

Briefly, 160 µL of ABTS+ solution were added to 40 µL of sample solution at different concentrations. After 10 min, the absorbance was measured at 734 nm. BHT, α-tocopherol, and quercetin were used as antioxidant standards. The results were expressed in terms of IC_{50} (The concentration of extract required to inhibit 50% of ABTS radical concentration).

2.7.3. Cupric Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC antioxidant capacity of PLFO and its USM were measured using the method described by Apak et al. [33]. For this reason, 40 µL of each sample solution were added to the premixed reaction mixture containing 60 µL of $CuCl_2$ (10 mM), 50 µL of neocuproine (7.5 mM), and 50 µL of CH_3COONH_4 buffer (1 M, pH 7.0).

After 1 h at room temperature, absorbance against a reagent blank was measured at 450 nm. BHT and BHA were used as standards, and the results were given as $A_{0.50}$ (The concentration providing 0.5 of absorbance at 450 nm).

2.8. Assessment of antidiabetic activity

2.8.1. α-amylase inhibition assay

α-amylase inhibitory activity was performed using the Zengin et al. [34] method with minor modifications. In a 96-well microplate, a volume of 25 µL of each sample solution was mixed with 50 µL of α-amylase solution (1U) in phosphate buffer (pH 6.9) with 6 mM sodium chloride and incubated for 10 min at 37°C. After this first incubation, the reaction was initiated by the addition of 50 µL of starch solution (0.1%). The microplate was incubated for another 10 min at 37°C. The enzymatic reaction was then stopped by the addition of 25 µL of HCl (1 M), after which 100 µL of the iodine-potassium iodide solution were added. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution. The sample and blank absorbances were read at 630 nm. The blank absorbance was subtracted from sample one and acarbose was used as a positive control. The results were given as IC_{50} value corresponding to the concentration that gives 50% inhibition.

2.8.2. α-glucosidase inhibition assay

Yeast α-glucosidase inhibition assay was performed using the substrate p-nitrophenyl-α-D-glucopyranoside (pNPG) according to the method described by Lordan et al. [35] with some modifications.
In a 96-well microplate, 50 µL of the sample solution in 100 mM sodium phosphate buffer (pH 6.9) and 100 µL of 0.1 U/mL α-glucosidase in phosphate buffer were mixed and incubated for 5 min at 37°C. A volume of 50 µL of 5 mM of pNPG solution (in phosphate buffer) was then added to each well, and the reaction mixture was incubated at 37 °C for 20 min. The absorbance of the released 4-nitrophenol was measured at 405 nm. Acarbose was included as a positive control, while a negative control was prepared without a sample. The results were given as IC_{50} value corresponding to the concentration that gives 50% inhibition.

2.9. Assessment of neuroprotective activity

2.9.1. Acetylcholinesterase inhibition assay

Acetylcholinesterase (AChE) inhibitory activity was determined using the method of Ellman et al. [36]. Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution, and 20 µL of AChE (5.32×10-3 U) solution were mixed in a 96-well microplate and incubated for 15 min at 25°C. Afterward, 10 µL of 0.5 mM DTNB (5,5'-dithiobis (2-nitro-benzoic acid)) were added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.71 mM). The absorbance was recorded at a wavelength of 412 nm. Galantamine was used as a reference compound, and the results were given as IC_{50} value corresponding to the concentration that gives 50% inhibition.

2.9.2. Butyrylcholinesterase inhibition assay

The BChE inhibitory activity of PLFO and USM was operated according to the same method as AChE, by replacing the AChE with BChE enzyme (6.85×10^{-3} U) and the acetylthiocholine iodide with butyrylthiocholine chloride substrate (0.2 mM). The results were given as IC_{50} value corresponding to the concentration that gives 50% inhibition [36].

2.10. Statistical analyses

Results are presented as the mean ± SD of three measurements. The IC_{50} and A0.50 values were calculated by linear regression analysis, and one-way analysis of variance ANOVA using XLSTAT to detect significant differences between means ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Extraction yields

The oil obtained is greenish yellow, slightly viscous at room temperature, and freezing at low temperature. The extraction yield was 31% based on dry matter weight. This result is lower than that found by Ben Daoued et al. [7] (38%), who used the same method for the extraction of PLFO; this can be explained by the early ripening stage of our *P. lentiscus* L. fruits (October) compared with that of Ben Daoued et al. [7] (December).

The unsaponifiable fraction obtained from PLFO is bright yellow with a pasty consistency. The yield was 4%, this value is higher than that found by Boulebda et al. [16]; this can be explained by the difference in PLFO extraction methods.

3.2. TPC, TFC, and antioxidant activity

Polyphenols and flavonoids occurring ubiquitously in medicinal plants have a wide variety of biological properties, including antioxidant activity. This last has great importance in terms of oxidative stress caused by the overproduction of free radicals, leading to damage to biological molecules (lipids, proteins, and DNA). These damages are often responsible for chronic and degenerative diseases [37].

Since the pathogenesis of many diseases is accompanied by the production of free radicals that generate oxidative stress, the assessment of polyphenols and flavonoids content as well as the antioxidant activity of new plant species is therefore of great interest.

The amounts of total phenolic content and total flavonoid content of PLFO and USM are presented in Table 1. The values of TPC and TFC in PLFO are 25,19 ± 0,67 µg GAE/mg and 20,90 ± 4,41 µg QE/mg of PLFO respectively. These results are higher than those already described in the literature [7,11,38]. Regarding the USM, the amounts of TPC and TFC are 18,70 ± 2,89 µg GAE/mg and 12,5 ± 2,65 µg QE/mg of USM respectively. These results indicate that the USM isolated from PLFO contains a considerable amount of phenolic compounds. According to the bibliographic data, lentisc oil presents a higher polyphenol content than that of virgin argan oil, soybean oil, sunflower oil, and corn oil but lower than that of virgin olive oil [8].

Table 1. Total phenolic content and Total flavonoid content of PLFO and its USM

Sample	TPC (µg GAE/mg extract)[a]	TFC (µg QE/mg extract)[b]
PLFO	25,19 ± 0,67	20,90 ± 4,41
USM	18,70 ± 2,89	12,5 ± 2,65

TPC and TFC values are the mean ± SD of three parallel measurements (Tukey test, P ≤ 0.05).

[a]Total phenolics are expressed as µg Gallic acid equivalents/mg of extract

[b]Total flavonoids are expressed as µg Quercetin equivalents/mg of extract

Regarding the antioxidant activity of PLFO and USM, three different assays were used to evaluate the radical scavenging activities and the reducing power of the samples. Table 2 shows IC_{50} values and the concentrations of samples associated with the percentage of inhibition reached for DPPH and ABTS assays.

Table 2. Radical scavenging activity of PLFO and its USM.

	% Inhibition in DPPH assay							
	12.5 µg	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**IC_{50} µg/mL**
PLFO[a]	NA	NA	NA	NA	2,99 ±3,57	8,12±3,69	10,28±4,28	>800
USM[a]	NA	NA	NA	NA	NA	3,46±2,54	3,80±2,98	>800
BHA[b]	76,55± 0,48	79,89± 0,26	81,73±0,10	84,18±0,10	87,13±0,17	89,36±0,19	90,14±0,00	6.14±0.41
BHT[b]	49,09± 0,76	72,63± 2,06	88.73±0,89	94.00±0,31	94.97±0,08	95.38±0,41	95.02±0,23	12.99±0.41
α-Tocopherol[b]	37,21±1,82	81,53±1,51	89,23±0,12	89,38±0,19	89,45±0,22	89,99±0,23	89,52±0,33	13.02±5,17
% Inhibition in ABTS assay								
PLFO[a]	NA	NA	NA	NA	NA	NA	NA	NA
USM[a]	NA	NA	NA	NA	NA	10,50±3,36	30,25±3,22	>800
BHT[b]	69.21±0,40	78.23±1,34	88.12±1,28	88,76±3,07	90.85±1,74	90.95±0,51	96.68±0.39	1.29±0.30
BHA[b]	92.83±1,42	94.68±0,42	94.95±0,90	95.32±0,25	95.59±0,47	95.83±0,15	95,86±0,10	1.81±0.10

[a]Values expressed are means±S.D of three parallel measurements (Tukey test, P ≤ 0.05).

[b]*Reference compounds.*
BHT: Butylated hydroxytoluene
BHA: Butylatedhydroxyanisole, NA: not active.

Concerning the DPPH assay, a lower IC_{50} value indicates a higher activity. Both PLFO and USM showed low DPPH radical scavenging activity with an IC_{50} > 800µg/mL (Table 2.) compared with the reference BHA, BHT, and α-Tocopherol (6.14 µg/mL, 12.99 µg/mL, and 13.02 µg/mL respectively). Such a weak ability to scavenge free radical DPPH by PLFO has been previously described by Ben Daoued et al. [7] who reported an IC_{50} value of 5.34 mg/mL, and Belyagoubi-Benhammou et al. [8] who found that the IC_{50} was reached at a concentration of 20.61 mg/mL of the oil (4 mg/mL in our case).
The DPPH radical scavenging power varies with many factors including: 1) phenolic compounds, like flavonoids, react with the DPPH radical by hydrogen atom donation to free radicals, and/or single electron transfer [7]. 2) The stage of ripening and date of harvest: the antioxidant activity increases during the process of maturation, this is probably due to a proportional increase in the number of phenols [17]. 3) The site of harvest; and 4) The extraction method (pressing method or traditional one) [19]. However, in comparison with other edible oils reported in the literature, the DPPH radical scavenging activity of PLFO is higher than that of kenaf seed, corn, olive, rice bran, soybean, and palm oils [7]. This activity is also higher than that of other species from the genus *Pistacia* (*P. vera* seed oil) [19].

Concerning the ABTS assay, PLFO has not shown any activity, whereas the USM has displayed low inhibition activity (IC_{50} > 800 µg/mL) compared to BHT and BHA (with IC_{50} 1.29 and 1.81 µg/mL respectively).

On the other hand, PLFO exhibited a low CUPRAC reducing antioxidant capacity ($A_{0.50}$ >800 µg/mL) compared with the positive control BHA and BHT ($A_{0.50}$ of 5,35 and 8.97 µg/mL respectively), as shown in Table 3. However, the USM displayed moderate antioxidant activity with $A_{0.50}$ of 230,37 µg/mL. This finding supports the fact that the antioxidant activity of PLFO is mainly attributed to its USM. Furthermore, the antioxidant effect of the unsaponifiable fractions isolated from several vegetable oils has been reported in the literature [39,40,41].

Table 3. CUPRAC assay of PLFO and USM

Samples	Absorbances in CUPRAC assay							
	12.5 µg	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**$A_{0.50}$ (µg/mL)**
PLFO[a]	0,24±0,00	0,24±0,01	0,25±0,01	0,25±0,02	0,28±0,01	0,33±0,03	0,36±0,01	>800
USM[a]	0,25±0,00	0,26±0,01	0,30±0,01	0,37±0,00	0,49±0,01	0,66±0,01	0,88±0,01	230,37±6,32
BHA[b]	1,12±0,05	1,95±0,31	3,14±0,46	3,58±0,42	3,35±0,20	3,77±0,19	3,92±0,13	5,35±0,71
BHT[b]	1.41±0.03	2.22±0.05	2.42±0.02	2.50±0.01	2.56±0.05	2.86±0.07	3.38±0.13	8.97±3.94

[a]*Values expressed are means±S.D of three parallel measurements (Tukey test, P ≤ 0.05).*
[b]*Reference compounds.*
BHA: Butylatedhydroxyanisole
BHT: Butylated hydroxytoluene

3.3. Enzymes inhibitory activities

Several enzymes involved in key metabolic processes are considered important targets for the prevention or management of related health disorders. For instance, type 2 diabetes mellitus (T2D) is a metabolic disorder resulting from a deficiency in insulin secretion, insulin action, or both, affecting carbohydrate, fat, and protein metabolism. α-amylase and α-glucosidase are enzymes responsible for the breakdown of complex carbohydrates such as starch and glycogen into absorbable monosaccharides. One therapeutic option for treating T2D is to decrease postprandial blood glucose levels through the inhibition of the carbohydrates-hydrolyzing enzymes, α-amylase, and α-glucosidase, thereby retarding the absorption of glucose [42]. Acarbose and Miglitol are examples of such synthetic drugs that, however, can lead to gastrointestinal adverse effects [43]. It is for this reason that numerous researchers are focusing on plants as natural sources of these inhibitor molecules to better control postprandial hyperglycemia with minimal side effects. Several bioactive compounds and medicinal

plants were found to be potent inhibitors of α-amylase and α-glucosidase [42,44,45]. From the same viewpoint, the inhibition of acetylcholinesterase and butyrylcholinesterase is an important strategy for managing Alzheimer's disease (AD). This latter is a neurological disorder in which the death of brain cells causes memory loss, cognitive decline, and ultimate dementia. The deficit of cholinergic neurotransmission is one of the causes of AD. Currently, cholinesterase inhibitors are being used as the first-line treatment for AD. These molecules aim to promote cholinergic neurotransmission to treat memory disturbances [46]. For this purpose, several medicinal plants have been proven effective with fewer adverse effects [47,48]. In the present work, PLFO and USM were investigated for the first time for their *in vitro* inhibitory effects against key enzymes involved in type 2 diabetes mellitus and Alzheimer's disease.

The α-amylase inhibitory activity of both samples is presented in Table 4. The low IC_{50} value corresponds to a high inhibition power. According to the literature, the inhibitory effect of samples is classified as potent (>50% inhibition), moderate (30–50% inhibition), and inactive or showing low activity (<30% inhibition) [47]. Consequently, PLFO showed low α-amylase inhibitory activity (IC_{50} > 400). On the other hand, the USM showed a much higher inhibitory activity (with IC_{50} of 180.93 µg/mL) than that of acarbose used as a reference compound (IC_{50} of 3650,93 µg/mL).

Table 4. α-amylase inhibitory activity of PLFO and its USM.

Samples	**% inhibition α-amylase**							**IC_{50} (µg/ml)**
	6,25 µg	**12,5 µg**	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	
PLFO [a]	NA	NA	NA	8,84±1,30	11,95±2,53	15,42±2,18	18,33±1,60	**> 400**
USM [a]	23,82±4,88	34,45±5,42	37,14±1,77	41,53±3,45	43,63±4,00	53,37±2,28	61,94±3,70	**180,93± 41,41**
Acarbose [b]	**62,5 µg**	**125 µg**	**250 µg**	**500 µg**	**1000 µg**	**2000 µg**	**4000 µg**	**3650,93±10,70**
	7,76±0,17	8,08±0,30	9,46±0,11	10,70±0,96	31,81±2,89	37,21±3,54	53,05±1,59	

[a] *Values expressed are means ± S.D. of three parallel measurements (Tukey test, P ≤ 0.05).*
[b] *Reference compound.*
NA: not active.

This strong inhibitory activity of the USM against the α-amylase enzyme was higher with the fraction isolated from the oil than that observed with the whole oil, hence the importance of separating the unsaponifiable from the oil. This result is consolidated by a previous study that reported promising antidiabetic activity in vivo as well as in vitro (α-amylase inhibitory activity) of fruit extract of *P. lentiscus* L. [49].

Concerning the α-glucosidase inhibitory effect, as shown in Table 5, both PLFO and USM displayed a potent inhibitory power (with an IC_{50} of 136.47 and 155.77 µg/mL respectively) compared with acarbose (IC_{50} of 275.43 µg/mL).

Table 5. α-glucosidase inhibitory activity of PLFO and USM

Samples	**% inhibition α-glucosidase**							**IC_{50} (µg/ml)**
	15,625 µg	**31,25 µg**	**62,5 µg**	**125 µg**	**250 µg**	**500 µg**	**1000 µg**	
PLFO [a]	3,44±0,21	7,36±1,19	16,23±0,65	43,94±1,94	69,96±5,89	90,81±1,61	96,34±0,43	**136,47 ± 34,16**
USM [a]	16,28±7,23	30,75±3,14	33,01±4,07	45,65±1,10	66,07±3,04	85,74±8,53	99,21±0,53	**155,77 ± 15,54**
Acarbose [b]	**78,125 µg**	**156, 25 µg**	**312,5 µg**	**625 µg**	**1250 µg**	**2500 µg**	**5000 µg**	**275,43 ± 1,59**
	27,43±2,18	38,91±3,20	54,86±1,79	67,29±2,63	80,19±1,66	85,54±0,45	91,05±0,72	

[a] *Values expressed are means ± S.D. of three parallel measurements (Tukey test, P ≤ 0.05).*
[b] *Reference compound.*

PLFO and its USM showed barely the same inhibitory profile against α-glucosidase, which suggests that this effect is attributed to the USM contained in the oil. However, in this case, the separation of USM has not caused any difference in the inhibitory power.

Cholinesterase inhibition

The AChE and BChE inhibitory activities are illustrated in Tables 6 and 7. PLFO displayed a potent inhibitory effect against both enzymes with IC_{50} values of 68,06 and 99,57µg/mL respectively, compared to galantamine (IC_{50} of 6,27 and 34,75 µg/mL respectively). While USM showed a moderate inhibitory effect against both enzymes compared to the reference compound. Hence, the pronounced inhibitory activity of PLFO against both cholinesterases is mainly attributed to the whole oil.

Table 6. Acetylcholinesterase inhibitory activity of PLFO and UMS

Samples	% inhibition acetylcholinesterase							IC_{50} µg/mL
	3,125 µg	6,25 µg	12,5 µg	25 µg	50 µg	100 µg	200 µg	
PLFO[a]	12,91± 4,73	18,87± 6,80	33,64± 3,87	36,55± 4,63	44,75± 3,93	60,46± 6,48	77,25± 5,77	**68,06 ± 10,36**
USM[a]	NA	NA	NA	NA	5,34± 2,85	18,82± 2,80	36,82± 1,78	**>200**
Galantamine[b]	35,93 ± 2,28	43,77 ± 0.00	68,50 ± 0,31	80,69 ± 0,41	85,78 ± 1,63	91,80 ± 0,20	94,77 ± 0,34	**6,27±1,15**

[a] *Values expressed are means ± S.D. of three parallel measurements (Tukey test, P ≤ 0.05).*
[b] *Reference compound.*
NA: not active.

Table 7.Butyrylcholinesterase inhibitory activity of PLFO and UMS

Samples	% inhibition butyrylcholinesterase							IC_{50} µg/mL
	3,125 µg	6,25 µg	12,5 µg	25 µg	50 µg	100 µg	200 µg	
PLFO[a]	18,74± 6,87	26,86± 8,91	30,18± 3,15	41,35± 7,18	40,34± 0,47	51,04± 2,87	67,97± 1,69	**99,57± 7,60**
USM[a]	NA	NA	NA	20,29± 1,32	20,90± 6,10	29,23± 5,75	41,56± 8,39	**>200**
Galantamine[b]	3,26± 0,62	6,93± 0,62	24,03± 2,94	45,13± 2,60	63,87± 2,85	73,57± 0,77	78,95± 0,58	**34,75±1,99**

[a] *Values expressed are means ± S.D. of three parallel measurements (Tukey test, P ≤ 0.05).*
[b] *Reference compound.*
NA: not active.

Similar findings were reported by Ammari et al. [27], who evaluated the neuroprotective effect of PLFO against damage induced by lipopolysaccharide in rats. In addition, PLFO has been previously reported to contain considerable rates of fatty acids, particularly α-linolenic and palmitic acids, known to be good BChE inhibitors [50], and sterols, including β-sitosterol which possesses strong AChE inhibition [51].

4. CONCLUSION

In summary, this is the first report on the *in vitro* antidiabetic and neuroprotective effects of *Pistacia lentiscus* L. fatty oil and its unsaponifiable matter. The oil displayed a strong anticholinesterase effect, while the USM exhibited potent antidiabetic activity; both could be considered for the management of type 2 diabetes and Alzheimer's disease.

Overall, the data presented herein is promising for the ongoing research on natural remedies and bioactive compounds. Nevertheless, these findings are just preliminary and should be supported by further investigations *in vivo*. Additionally, studies on individual bioactive compounds are required to be more conclusive.

REFERENCES

1. Barazani O, Dudai N, Golan-Goldhirsh A. Comparison of Mediterranean *Pistacia lentiscus* Genotypes by Random Amplified Polymorphic DNA, Chemical, and Morphological Analyses. Journal of Chemical Ecology. 2003;29(8):1939-1952.

2. Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques et méridionales. Centre nationale de la recherche scientifique. Paris; 1963.

3. Chinou I, & Delbò M. Assessment report on *Pistacia lentiscus* L., resin (mastix). European Medicines Agency report. 2015. https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-pistacia-lentiscus-l-resin-mastic_en.pdf.

4. Bellakhdar J. Contribution à l'étude de la pharmacopée traditionnelle au maroc : la situation actuelle, les produits les sources du savoir. Doctoral thesis. University of METZ. France; 1997

5. Ait Youcef M. Plantes médicinales de Kabylie. Ibis press. France; 2006.

6. IUCN. A guide to medicinal plants in North Africa. IUCN Centre for Mediterranean Cooperation. Spain; 2005

7. Ben Daoued K, Chouaibi M, Gaout N, Bel Haj O, Hamdi S. Chemical composition and antioxidant activities of cold pressed lentisc (*Pistacia lentiscus* L.) seed oil. La rivista italiana delle sostanze grasse. 2015;93:31-38.

8. Belyagoubi-Benhammou N, Belyagoubi L, El Zerey-Belaskri A, Zitouni A, Ghembaza N, Benhassaini H et al. Fatty acid composition and antioxidant activity of *Pistacia lentiscus* L. fruit fatty oil from Algeria. Journal of Food Measurement and Characterization. 2018;12:1408–1412.

9. Mezni F, Khouja M L, Gregoire S, Martine L, Khaldi A, Berdeaux O. Effect of a growing area on tocopherols, carotenoids, and fatty acid composition of *Pistacia lentiscus* edible oil. Natural Product Research. 2014a;28(16):1225-1230.

10. Mezni F, Labidi A, Khouja M L, Martine L, Berdeaux O, Khaldi A. Diversity of Sterol Composition in Tunisian *Pistacia lentiscus* Seed Oil. Chem. Biodiversity. 2016;13:544–548.

11. Mezni F, Slama A, Ksouri R, Hamdaoui G, Khouja M L, Khaldi A. Phenolic profile and effect of growing area on *Pistacia lentiscus* seed oil. Food chemistry. 2018;257:206–210.

12. Boukeloua A, Belkhiri A, ,Yilmaz M A, Temel H, Sabatini S. Chemical profiling and total thickness-excised wound-healing activity of *Pistacia lentiscus* L. fruits growing in Algeria. Cogent Biology. 2016;2(1).

13. Djerrou Z, Maameri Z, Hamdi-Pacha Y, Serakta M, Riachi F, Djaalab H et al. Effect of virgin fatty oil of *pistacia lentiscus* on experimental burn wound's healing in rabbits. African Journal of Traditional CAM. 2010;7(3):258–263.

14. Maameri Z, Beroual K, Djerrou Z, Habibatni S, Benlaksira B, Serakta M et al. Preliminary study to assess the cicatrizing activity of honey and *Pistacia lentiscus* fatty oil mixture on experimental burns in rabbits. International Journal of Medicinal and Aromatic Plants. 2012;2:476-480.

15. Ben Khedir S, Bardaa S, Chabchoub N, Moalla D, Sahnoun Z, Rebai T. The healing effect of *Pistacia lentiscus* fruit oil on laser burn. Pharmaceutical Biology. 2017;55(1):1407–1414.

16. Boulebda N, Belkhiri A, Belfadel F, Bensegueni A. Dermal Wound Healing Effect of *Pistacia Lentiscus* Fruit's Fatty Oil. Pharmacognosy Research. 2009;1(2):66-71.

17. Mezni F, Labidi A, Msallem M, Boussaid M, Khouja M L, Khaldi A. Influence of harvest date on fatty acid composition and antioxidant activity of *Pistacia lentiscus* L. edible oils. Journal of Materials and Environmental Science. 2014b;5(6):1703-1708.

18. Ben Khedir S, Moalla D, Jardak N, Mzid M, Sahnoun Z, Rebai T. *Pistacia lentiscus* fruit oil reduces oxidative stress in human skin explants caused by hydrogen peroxide. Biotechnic & Histochemistry. 2016;91(7):480-491.

19. Mezni F, Maaroufi A, Labidi A, Msallem M, Boussaid M, Khouja M L et al. Fatty acid composition, antioxidant and antibacterial activities of *Pistacia lentiscus* L. fruit oils. Journal of Medicinal Plants Research. 2012;6(39):5266-5271.

20. Mezni F, Aouadhi C, Khouja M L, Khaldi A, Maaroufi A. *In vitro* antimicrobial activity of *Pistacia lentiscus* L. edible oil and phenolic extract. Natural Product Research. 2014c;29(6):565-570.

21. Orrù G, Demontis C, Mameli A, Tuveri E, Coni P, Pichiri G et al. The Selective Interaction of *Pistacia lentiscus* Oil vs. Human Streptococci, an Old Functional Food Revisited with New Tools. 2017;24(8):2067.

22. Abidi A, Aissani N, Sebai H, Serairi R, Kourda N, Ben Khamsa S. Protective Effect of *Pistacia lentiscus* Oil Against Bleomycin-Induced Lung Fibrosis and Oxidative Stress in Rat. Nutrition and cancer. 2017;69(3):490-497.

23. Chekchaki N, Rouibah Z, Rouag M, Messarah M. Effets de *Pistacia lentiscus* sur les variations des niveaux d'interleukine-4 et de protéines totales sériques et dans le liquide du lavage bronchoalvéolaire dans un modèle d'asthme expérimental. Revue Française d'Allergologie. 2016;56(3):302. French.

24. Mezni F, Shili S, Ben Ali N, Khouja M L, Khaldi A, Maaroufi A. Evaluation of *Pistacia lentiscus* seed oil and phenolic compounds for in vitro antiproliferative effects against BHK21 cells. Pharmaceutical Biology. 2015;54(5):747-751.

25. Djerrou Z. Anti-hypercholesterolemic effect of *Pistacia lentiscus* fatty oil in egg yolk-fed rabbits: a comparative study with simvastatin. Chinese Journal of Natural Medicines. 2014;12(8):561-566.

26. Boutemine I-M, Amri M, Amir Z-C, Fitting C, Mecherara-Idjeri S, Layaida K et al. Gastro-protective, therapeutic and antiinflammatory activities of *Pistacia lentiscus* L. fatty oil against ethanol-induced gastric ulcers in rats. Journal of Ethnopharmacology. 2018;5(224):273-282.

27. Ammari M, Othman H, Hajri A, Sakly M, Abdelmelek H. *Pistacia lentiscus* oil attenuates memory dysfunction and decreases levels of biomarkers of oxidative stress induced by lipopolysaccharide in rats. Brain research bulletin. 2018;140:140-147.

28. Council of Europe, European Pharmacopoeia Commission, European Directorate for the Quality of Medicines & Healthcare. European pharmacopoeia. 7th ed. Council Of Europe : European Directorate for the Quality of Medicines and Healthcare, Strasbourg; 2010.

29. Müller L, Gnoyke S, M. Popken A, Böhm V. Antioxidant capacity and related parameters of different fruit formulations. LWT - Food Science and Technology. 2010;43(6):992-999.

30. Topçu G, Ay M, Bilici A, Sarıkürkcü C, Öztürk M, Ulubelen A. A new flavone from antioxidant extracts of *Pistacia terebinthus*. Food Chemistry. 2007;103(3):816-822.

31. Blois M. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958;181: 1199–1200.

32. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology & medicine. 1999;26(9-10):1231-7.

33. Apak R, Güçlü K, Oztürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of agricultural and food chemistry. 2004;52(26):7970-81.

34. Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R, Ceylan O. A comprehensive study on phytochemical characterization of *Haplophyllum Trifolium* Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer's, skin diseases, and type II diabetes. Industrial Crops and Products. 2014a;53:244-251.

35. Lordan S, Smyth T J, Soler-Vila A, Stanton C, Ross R P. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. Food chemistry. 2013;141(3):2170-6.

36. Ellman GL, Courtney KD, Andres V Jr, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. 1961;7:88-95.

37. Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R. *Sideritis galatica* Bornm.: A source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. Journal of Functional Foods. 2014b;11:538-547.

38. Bouyahya A, Dakka N, Talbaoui A, El Moussaoui N, Abrini J, Bakri Y. Phenolic contents and antiradical capacity of vegetable oil from *Pistacia lentiscus* (L). Journal of Materials and Environmental Sciences. 2018;9(5):1518-15.

39. Ghorbel I, Khemakhem M, Boudawara O, Marrekchi R, Jamoussi K, Ben Amar R et al. Dietary unsaponifiable fraction of extra virgin olive oil supplementation attenuates lung injury and DNA damage of rats co-exposed to aluminum and acrylamide. Environmental science and pollution research international. 2016;23(19):19397-408.

40. Farhoosh R, Tavassoli-Kafrani M H, Sharif A. Antioxidant activity of the fractions separated from the unsaponifiable matter of bene hull oil. Food Chemistry. 2011;126(2):583-589.

41. Tavakoli J, Estakhr P, Zarei Jelyani A. Effect of unsaponifiable matter extracted from Pistacia khinjuk fruit oil on the oxidative stability of olive oil. Journal of Food Science and Technology. 2017;54(9):1-9.

42. Sales PM, Souza PM, Simeoni LA, Silveira D. α-Amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source. Journal of pharmacy & pharmaceutical sciences. 2012;15(1):141-83.

43. Vujanović M, Zengin G, Đurović S, Mašković P, Cvetanović A, Radojković M. Biological activity of extracts of traditional wild medicinal plants from the Balkan Peninsula. South African Journal of Botany. 2018;120:213-218.

44. Etxeberria U, de la Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha-amylase. Expert opinion on therapeutic targets. 2012;16(3):269-297.

45. Hamza N, Berke B, Umar A, Cheze C, Gin H, Moore N. A review of Algerian medicinal plants used in the treatment of diabetes. Journal of Ethnopharmacology. 2019;238:111841.

46. Bhushan I, Kour M, Kour G, Gupta S, Sharma S, Yadav A. Alzheimer's disease: Causes & treatment – A review. Annals of Biotechnology. 2018;1(1):1002.

47. Vinutha B, Prashanth D, Salma K, Sreeja S L, Pratiti D, Padmaja R et al. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. Journal of Ethnopharmacology. 2007;109(2):359-363.

48. Ahmed-Chaouch M, Cheriet T, Beretta G, Sarri D, Bensouici C, Ouelbani R et al. Chemical composition, in vitro antioxidant, anticholinesterase, and antibacterial activities of Linaria scariosa Desf. Natural Product Research. 2021;35(10):1722-1726.

49. Mehenni C, Atmani Kilani D, Dumarçay S, Perrin D, Gérardin P, Atmani D. Hepatoprotective and antidiabetic effects of *Pistacia lentiscus* leaf and fruit extracts. Journal of Food and Drug Analysis. 2016;24(3):653-669.

50. Öztürk M, Tel G , Öztürk F A, Duru M E. The Cooking Effect on Two Edible Mushrooms in Anatolia: Fatty Acid Composition, Total Bioactive Compounds, Antioxidant and Anticholinesterase Activities. Records of natural products. 2014;8(2):189-194.

51. Ayaz M, Junaid M, Ullah F, Subhan F, Sadiq A, Ali G, Ovais M, Shahid M, Ahmad A, Wadood A, El-Shazly M, Ahmad N, Ahmad S. Anti-Alzheimer's Studies on β-Sitosterol Isolated from *Polygonum hydropiper* L. Frontiers in pharmacology. 2017;8:697.