Journal of Pharmaceutical Research International

Volume 35, Issue 15, Page 11-23, 2023; Article no.JPRI.100445
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Pistacia lentiscus L. Fatty Oil and its Unsaponifiable Matter: Antidiabetic and Neuroprotective Activities

Kaouthar Hamdi [a*], Nadia Boughandjioua Amoura [b], Amira Noui [c], Farid Dalia [a], Meryem Siline [a] and Abdelmalik Belkhiri [a]

[a] Department of Pharmacy, Faculty of Medicine, Laboratory of Pharmacognosy, Constantine III University, Algeria.
[b] Department of Pharmacy, Faculty of Medicine, Laboratory of Pharmacognosy, Annaba University, Algeria.
[c] Biotechnology Research Center, Constantine, Algeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2023/v35i157376

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/100445

Original Research Article

Received: 25/03/2023
Accepted: 27/05/2023
Published: 05/06/2023

ABSTRACT

Background: The inhibition of key enzymes involved in type II diabetes and Alzheimer's disease is an effective therapeutic approach for managing these health disorders. This study aimed to assess the antidiabetic and neuroprotective effects of *Pistacia lentiscus* L.
Methods: The potential of *Pistacia lentiscus* L. fatty oil (PLFO) was investigated against the activities of α-amylase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase. Additionally, the unsaponifiable matter (USM) of this oil was isolated and screened for the same activities. Furthermore, the antioxidant power of both extracts was evaluated using DPPH, ABTS, and CUPRAC assays.

**Corresponding author: E-mail: hamdikaouthar19@gmail.com;*

Results: The results revealed that PLFO exhibited a low antioxidant effect primarily due to its USM. The potent antidiabetic effect of PLFO was also attributed to its USM, while the whole oil was responsible for the neuroprotective effect.
Conclusion: These findings suggest that PLFO and its USM could serve as natural sources of compounds for managing 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 emergence of innovative trends in the pharmaceutical fields and medication, many populations worldwide still rely on traditional medicine to fulfill their healthcare needs. In Algeria, as well as in numerous other African countries, traditional medicine is often the primary choice for treating various health conditions. Based on cultural and natural heritage, people relying on traditional medicine believe that *Pistacia lentiscus* L. has consistently demonstrated efficacy in managing common diseases.

This plant, locally known as "Derou, is one of the most common shrubs found in the Mediterranean regions and yields fatty oil commonly used for treating wounds and burns. It exhibits significant morphological and chemical variability as well as important genotypic polymorphism [1,2]. The most renowned variety is undeniably the mastic tree, found on Chios island in Greece, which provides the highly regarded mastic of Chios (also known as Chios Gum Mastic or mastiha). This mastic is well-known worldwide for its multiple biological activities and applications [3].

The variety thriving in Algeria produces a type of resin, usually in a pasty or liquid, that does not harden like mastic [4]. However, this variety is primarily utilized for extracting valuable fatty oil, which has a long history in the folk medicine of many regions in the country. Using traditional tools, the oil is extracted from the black ripe fruits of *Pistacia lentiscus* L. and is employed as a traditional remedy to alleviate gastralgia, diarrhea and also as a food oil. Additionally, the oil is locally applied to treat wounds, scabies and rheumatism [5,6].

Recent studies have revealed that PLFO (*Pistacia lentiscus* L. fatty oil) primarily consists of fatty acids, with monounsaturated fatty acids (52%) being the predominant type. Polyunsaturated fatty acids account for approximately 18% of the oil and are mainly represented by linoleic acid. The oil also contains sterols, tocopherols, carotenoids, and chlorophyll [7-10]. Furthermore, this oil is considered a rich source of phenolic compounds, with nearly 40 compounds identified, including Gallic acid, Tyrosol, Vanillic Acid, Kaempferol, and others [7,11].

Numerous authors have highlighted the biological activities of PLFO. The oil has been reported to possess wound-healing and burn-healing effects [12-15]. The latter effect has been attributed to the unsaponifiable matter isolated from the oil [16]. Moreover, the oil exhibits antioxidant activity [7,17,18], antibacterial properties [19-21], and antifungal activities [20]. Many other properties have been attributed to this oil including anti-inflammatory effects [22,23], antiasthmatic properties [23], antiproliferative activity [24], anti-hypercholesterolemic effects [25], as well as gastroprotective and antiulcerogenic effects [26].

Additionally, the oil has been found to prevent learning and memory disorders induced by lipopolysaccharide, and it attenuates oxidative damage in brain tissue and liver in rats through its 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 sufficient attention from researchers [16]. Thus, the present work represents 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 shaded and ventilated place.

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

2.2 Extraction of *Pistacia lentiscus* L. Fatty Oil (PLFO)

One kilogram of dried fruits of *P. lentiscus* L. was pressed at room temperature (20°C) using a cold-pressing machine (XING FA). The 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 described in the European Pharmacopoeia, 7th edition, with modifications as proposed by Boulebda [28,16].

Fifty grams 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 was achieved (1 h). After cooling, the ethanol was evaporated under vacuum using a rotary evaporator. Then, 100 mL of distilled water were added to the residue and transferred to a separatory funnel, where the liquid was carefully shaken with three portions of 150 mL each of 150 mL of diethyl ether. The ether layers were combined and dried over anhydrous sodium sulfate (Na2SO4). The ether was finally removed to recover the unsaponifiable fraction, which was stored at 4°C until use.

2.4 Sample Preparation and Spectral Measurements

Before each assay, stock solutions of each extract were prepared. Four milligrams of PLFO and four milligrams of USM were dissolved in 1 mL of methanol and shaken in a vortex. Each stock solution was diluted to obtain seven concentrations. For the determination of Total Polyphenolic Content (TPC) and Total Flavonoid Content (TFC), the concentration of stock solutions was adjusted to 1 mg/mL. All assays were performed in a 96-well microplate, and the absorbances were measured using a 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 the microplate assay method described by Müller et al. [29].

Twenty microliters of each aqueous extract (PLFO and USM at 1 mg/mL) were mixed with 100 µL of 1:10 diluted Folin-Ciocalteu reagent and 75 µL 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 using a microplate (Perkin Elmer Enspire, Singapore). Gallic acid was used as a standard for calibration and construction of a linear regression line, and water was used as a 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. Fifty microliters 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 using a 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 was determined spectrophotometrically using the DPPH assay [31]. α-tocopherol, BHT, and BHA were used as antioxidant standards. To 40 µL of sample solution at different concentrations, 160 µL of freshly prepared DPPH methanol solution were added. After incubation for 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 a 10-minute incubation, 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 was measured using the method described by Apak et al. [33]. For this assay, 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 incubation for 1 hour at room temperature, the 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 method described by Zengin et al. [34] with minor modifications. In a 96-well microplate, 25 µL of each sample solution were mixed with 50 µL of α-amylase solution (1U) in phosphate buffer (pH 6.9) containing 6 mM sodium chloride and incubated for 10 min at 37°C. After the initial incubation, the reaction was initiated by adding 50 µL of starch solution (0.1%). The microplate was incubated for an additional 10 min at 37°C. The enzymatic reaction was then stopped by adding 25 µL of HCl (1 M), followed by the addition of 100 µL of the iodine-potassium iodide solution. Similarly, a blank was prepared by adding sample solution to all reaction reagents without the enzyme solution. The absorbance of the sample and blank were read at 630 nm. The blank absorbance was subtracted from sample absorbance, and acarbose was used as a positive control. The results were reported as the IC_{50} value, which corresponds to the concentration that gives 50% inhibition.

2.8.2 α-glucosidase inhibition assay

The 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. Then, 50 µL of 5 mM of pNPG

solution (in phosphate buffer) were 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 reported as IC_{50} value, which corresponds 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, in a 96-well microplate, 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 and incubated for 15 min at 25°C. Then, 10 µL of 0.5 mM DTNB (5,5'-dithiobis (2-nitro-benzoic acid)) were added. The reaction was then initiated by adding 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 reported as the IC_{50} value, which corresponds to the concentration that gives 50% inhibition.

2.9.2 Butyrylcholinesterase inhibition assay

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

2.10 Statistical Analyses

The results are presented as the mean ± SD of three measurements. The IC_{50} and A0.50 values were calculated using linear regression analysis. One-way analysis of variance (ANOVA) was performed using XLSTAT to identify 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 solidifies at low temperatures. The extraction yield based on the dry matter weight was determined to be 31%. This result is lower than the extraction yield reported by Ben Daoued et al. [7] (38%) using the same extraction method for PLFO. The difference can be attributed to the early ripening stage of *P. lentiscus* L. fruits used in our study (harvested in October), compared to the fruits used by Ben Daoued et al. [7] (harvested in December).

The unsaponifiable fraction obtained from PLFO has a bright yellow color and a pasty consistency. The yield of the unsaponifiable fraction was determined to be 4%, which is higher than the yield reported by Boulebda et al. [16]. This difference can be attributed to variations in the extraction methods used for obtaining the PLFO.

3.2 TPC, TFC, and Antioxidant Activity

Polyphenols and flavonoids, which are present in medicinal plants, possess various biological properties, including antioxidant activity. This last is of great importance in combating oxidative stress caused by the overproduction of free radicals, which can lead to damage to biological molecules such as lipids, proteins, and DNA. Such damage is often associated with 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 total phenolic content (TPC) and total flavonoid content (TFC) of PLFO and its unsaponifiable fraction (USM) are presented in Table 1. The TPC and TFC values for PLFO determined to be 25,19 ± 0,67 µg GAE/mg and 20,90 ± 4,41 µg QE/mg of PLFO, respectively. These results are higher than those reported in the literature [7,11,38]. In the case of the USM, the amounts of TPC and TFC values were found to be 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 significant amount of phenolic compounds. According to the available literature, lentisk oil exhibits a higher polyphenol content compared to 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

In order to assess the antioxidant activity of PLFO and its USM, three distinct assays were employed to evaluate the radical scavenging activities and the reducing power of the samples. Table 2 presents IC_{50} values and the concentrations of the samples corresponding to the percentage of inhibition achieved in the DPPH and ABTS assays.

Regarding the DPPH assay, a lower IC_{50} value indicates higher activity. Both PLFO and USM exhibited low DPPH radical scavenging activity, with IC_{50} values greater than 800µg/mL (Table 2), compared with the reference BHA, BHT, and α-Tocopherol which demonstrated an IC_{50} values of 6.14 µg/mL, 12.99 µg/mL, and 13.02 µg/mL, respectively. The weak ability of PLFO to scavenge the DPPH free radical 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 is influenced by various factors, including the presence of phenolic compounds such as flavonoids, which can donate hydrogen atoms to free radicals or undergo single electron transfer [7]. Additionally, the stage of ripening and date of harvest can affect the antioxidant activity, likely due to an increase in phenol content as the fruit matures [17]. Other factors that may influence DPPH radical scavenging activity include the site of harvest, and the extraction method used such as pressing or traditional methods [19]. However, compared to other edible oils mentioned in the literature, PLFO exhibits higher DPPH radical scavenging activity than kenaf seed, corn, olive, rice bran, soybean, and palm oils [7]. Furthermore, PLFO demonstrates greater activity than other species from the Pistacia genus, such as *P. Vera* seed oil [19].

Regarding the ABTS assay, PLFO did not show any activity, while USM displayed low inhibition activity with an IC_{50} value greater than 800 µg/mL, in contrast to BHT and BHA which exhibited IC_{50} values of 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 compounds BHA and BHT which showed $A_{0.50}$ values of 5.35 and 8.97 µg/mL, respectively (Table 3). However, USM demonstrated moderate antioxidant activity with an $A_{0.50}$ value of 230,37 µg/mL. This finding supports the notion that the antioxidant activity of PLFO is primarily attributed to its USM. Additionally, the literature reports the antioxidant effect of the unsaponifiable fractions isolated from various vegetable oils [39-41].

3.3 Enzymes Inhibitory Activities

Several enzymes involved in crucial metabolic processes are considered significant targets for the prevention or management of related health disorders. Type II diabetes mellitus (T2D), for instance, 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 synthetic drugs that can achieve this, but they may also lead to gastrointestinal adverse effects [43]. Therefore, numerous researchers are focusing on plants as natural sources of inhibitor molecules to better control postprandial hyperglycemia with minimal side effects. Several bioactive compounds and medicinal plants have been found to be potent inhibitors of α-amylase and α-glucosidase [42,44,45]. Similarly, inhibiting acetylcholinesterase and butyrylcholinesterase is an important strategy for managing Alzheimer's disease (AD). AD is a neurological disorder

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

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

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.

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.*

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.

characterized by the death of brain cells, leading to memory loss, cognitive decline, and eventually dementia. Cholinergic neurotransmission deficits are one of the causes of AD. Currently, cholinesterase inhibitors are used as the first-line treatment for AD. These molecules aim to promote cholinergic neurotransmission to treat memory disturbances [46]. In this regard, several medicinal plants have proven effective with fewer adverse effects [47,48]. In the present study, PLFO and USM were investigated for the first time for their *in vitro* inhibitory effects against key enzymes involved in type II diabetes mellitus and Alzheimer's disease.

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

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, highlighting the importance of separating the unsaponifiable from the oil. This finding is supported by a previous study that reported promising *in vivo* antidiabetic activity as well as *in vitro* α-amylase inhibitory activity of the fruit extract of *P. lentiscus* L. [49].

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

Both PLFO and its USM exhibited a similar inhibitory profile against α-glucosidase, suggesting that this effect is attributed to the presence of USM in the oil. However, in this case, the separation of USM did not result in any difference in the inhibitory power.

3.4 Cholinesterase Inhibition

The inhibitory activities of AChE and BChE are presented in Tables 6 and 7, respectively. PLFO demonstrated 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). On the other hand, USM exhibited a moderate inhibitory effect against both enzymes compared to the reference compound. Therefore, the pronounced inhibitory activity of PLFO against both cholinesterases is primarily attributed to the whole oil.

Similar findings were reported by Ammari et al. [27], who investigated the neuroprotective effect of PLFO against lipopolysaccharide-induced damage in rats. Previous studies have also indicated that PLFO contains significant amounts of fatty acids, particularly α-linolenic and palmitic acids, which are known to be effective BChE inhibitors [50], as well as sterols such as β-sitosterol, which possesses strong AChE inhibitory effect [51].

4. CONCLUSION

In conclusion, this study provides the first evidence of the *in vitro* antidiabetic and neuroprotective effects of *Pistacia lentiscus* L. fatty oil and its unsaponifiable matter. The oil demonstrated potent anticholinesterase activity, while the USM exhibited significant antidiabetic activity, making them potential candidates for the management of type II diabetes and Alzheimer's disease. Overall, the findings presented in this study are promising for ongoing research on natural remedies and bioactive compounds. However, these results are preliminary and further *in vivo* investigations are necessary to support these findings. Moreover, studies focusing on individual bioactive compounds are warranted to draw more conclusive insights.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to thank Pr. Zahia KABOUCHE for her help in the oil extraction as well as Mr. BELADJINE M.A for the correction of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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. Available: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. 2014;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. 2014;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. 2014;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.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/100445