

Pistacia lentiscus L. oil from the sub-arid region of Hodna (Algeria): Influence of oil extraction process on phenolic content, scavenging power, antibacterial and antifungal activities

Lamia Derradji^{1,2*}, Abderrahim Benkhalel^{1,3*}, Kılılcım Yıldız⁴, Ahmed Nouasri⁵, Souhila Bouaziz Terrachet⁶, Zeynep Aksoylu Özbek⁴, Pelin Günç Ergönül⁴

Department of Microbiology and Biochemistry, Faculty of Sciences, University of M'sila, Algeria¹
Laboratory of Biology: Applications in Health and Environment, Faculty of Sciences, University of M'sila, Algeria²

Laboratory of Biodiversity and Biotechnological Techniques for the Valorization of Plant Resources, Faculty of Sciences, University of M'sila, Algeria³

Engineering Faculty, Department of Food Engineering, Manisa Celal Bayar University, Turkey⁴

Laboratory of Bioactive Products and Biomass Valorization Research, ENS Kouba, Algeria⁵

Applied Chemistry and Materials Laboratory (LabCAM), Faculty of Sciences, University of Mohamed Bouguerra, Algeria⁶

Corresponding author: 1,2* & 1,3*



Keywords:

Pistacia lentiscus oil, Hodna sub arid origin, Extraction process, phenolic content, scavenging power, antimicrobial activity

ABSTRACT

This study aimed to evaluate, for the first time, the phenolic contents, antioxidant, antibacterial, and antifungal properties of the Hodna sub-arid lentisk seed oils obtained through hot (traditional method) and cold extraction techniques. The cold pressing process resulted in a much greater yield (15%) compared to the traditional method (5%). Both Hodna lentisk oils obtained by cold pressing (HLCO) and hot extraction (HLHO) were rich in polyphenols, but the total phenolic content of HLCO (94.54 mg GAE/100 g) was twice that of HLHO (48.02 mg GAE/100 g), making HLCO a richer source of phenolic compounds. HLHO and HLCO showed very high free radical scavenging activity, which were respectively 113.81 and 107.47 mM TE/100 g. Limited inhibitory effects were observed against a panel of five pathogenic bacteria and six fungal strains. The sub-arid origin of *P. lentiscus* oils, more particularly the cold-extracted one, may be a natural source of promising phenolics compounds that could be incorporated into new products or replace synthetic compounds in the health, food, and cosmetics sectors.

DOI:

03.13478/Ama.28.03.2025.01



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1. Introduction

Pistacia lentiscus is the most widely used plant of the *Pistacia* genus in various parts of the world. *P. lentiscus*

is an evergreen shrub of the Anacardiaceae family, also known as the mastic tree. This plant is worldwide known for its various therapeutic properties [1- 3]. It grows in various soil types, including wastelands and shrublands, in the Mediterranean basin. The mastic tree is widely distributed in the “extreme” ecosystems of the Mediterranean basin and exhibits salinity resistance [4- 6]. *P. lentiscus*, a key component of mixed forests in the Mediterranean soil, has a broad geographical and bioclimatic distribution that ranges from humid to arid locations [7].

P. lentiscus has been used for about 5000 years [8]. Various parts of the plant may be utilized, including oil, resin, leaves, fruit, and aerial parts. Among these, the oil stands out as the most frequently used one. In Algeria, lentisk fixed oil serves as a significant therapeutic product with high potential economic value in forest areas. The fruit oil is valued for its beneficial properties serving as diuretic [8], an anti-inflammatory [9], an antiseptic [10], a healing agent for local skin diseases [11], burns, wounds [12], [13], gastric ulcers [14], coughs and colds [15]. *P. lentiscus* is considered an oleaginous plant [16]. Lentisk oil can be extracted through different methods. The cold-press method, which requires less energy and is environmentally friendly, has been utilized to extract oil from diverse seeds, while the classic approach (hot extraction) is the oldest and most prevalent one [17].

The purpose of this research is to investigate the influence of the sub-arid origin of *P. lentiscus* and the extraction process (traditional hot and cold methods) on the phenolic content, antioxidant and antimicrobial properties against five pathogenic bacteria and six fungal strains.

2. Materials and Methods

2.1 Standards and reagents

Folin-Ciocalteu, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and sodium carbonate, was obtained from Merck (Darmstadt, Germany). Mueller-Hinton agar and Sabouraud dextrose agar were obtained from Pasteur Institute (Algeria).

2.2 Plant material

The ripe fruits of *P. lentiscus* (Fig.1) were collected at full maturity (identical red color of seeds) in December, from the forest of El Hauran of the Hodna region (sub-arid region in the Northeast of Al-geria) (Fig.2). A relative voucher specimen was deposited and registered in the herbarium of the Uni-versity of M'sila, Algeria. The fruits were cleaned and air-dried in the shade at ambient temperature until obtaining a constant weight.



Fig. 1 Ripe fruits of *P. lentiscus*

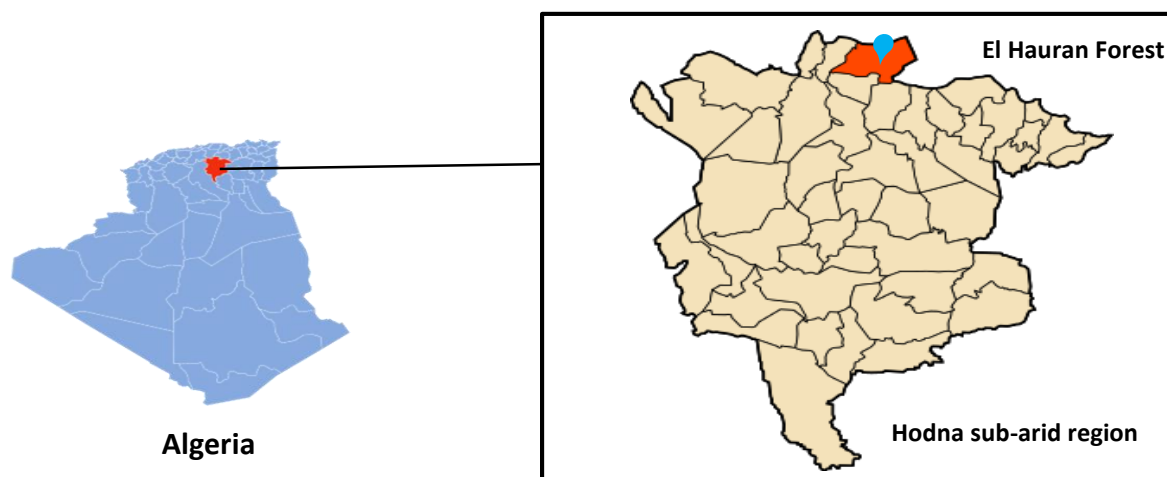


Fig. 2 Location of the study area

2.3 Extraction process

2.3.1 Hot extraction (traditional method)

In the hot extraction method, the fruits were ground into fine powder, boiled for 30 minutes with hot water, distributed over fiber disks, and subsequently pressed. Hot water was run down the sides of the disks to enhance oil filtration. The resulting oil (HLHO) was separated from the water through de-cantation and stored in flasks kept at 4°C away from light until use [13].

2.3.2 Cold extraction (pressing method)

In this method, the extraction was conducted using an industrial technique known as cold pressing extraction. A COMAF type screw press with a 5 kg/h capacity was utilized. This method is often de-fined as a solid-liquid phase separation system. The seeds were cleaned from their impurities before pressing. The crude oil (HLCO) obtained is then purified by sedimentation and filtration, and the cakes are removed. The oil was stored in amber-colored flasks at 4°C. The extraction yield, expressed as a function of dry matter, is calculated using the following formula:

$$\text{Yield}(\% \text{ DM}) = \frac{\text{Weight of the collected oil}}{\text{Dry weight of sample}} \times 100$$

2.4 Determination of total phenols content

The Folin-Ciocalteu assay was used for determining the total phenol concentration, with minor modifications [18]. Briefly, 0.5 g of oil dissolved in 10 mL of methanol; was mixed and centrifuged for 20 minutes at 6000 rpm. After recovering the methanolic phase, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent were added. After a 5 min incubation under dark conditions, 1 mL Na₂CO₃ solution (35%, w/v) was added. Finally, the mixture was diluted to 25 mL with distilled water, stirred, and left to stand for 120 minutes. The absorbance was measured at 725 nm using a microplate reader (Thermo Scientific Multiskan Go, USA). The results were expressed as milligrams of gallic acid equivalents per 100 g of oil (mg GAE/100 g oil) using a gallic acid calibration curve ($R^2 = 0.993$).

2.5 Trolox equivalent antioxidant capacity

The DPPH-radical scavenging assay developed by [19] was used to evaluate the oils' overall antioxidant capacity. A 0.5 g oil sample was weighed and dissolved in 10 mL of methanol. The mixture was vortexed and centrifuged at 6000 rpm for 20 min. The mixture was then made up of 0.2 mL of methanolic phase and 3.8 mL of methanolic DPPH solution (100 µM). Absorbance was measured using a spectrophotometer

operating at 517 nm after a 15-min incubation at 25°C in the dark. The results were reported as millimoles of Trolox equivalent per 100g of oil (mM TE/100g oil) using a Trolox calibration curve ($R^2 = 0.989$).

2.6 Antimicrobial activity

2.6.1 Microbial strains

The antibacterial activity was tested against a panel of five pathogenic bacteria including three Gram positive: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579) and *Listeria monocytogenes* (ATCC 7644) and two Gram negative *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853).

The antifungal activity was determined against five fungi: *Umbelopsis ramanniana* (NRRL 1829), *Fusarium culmorum* (NRRL.1829), *Aspergillus flavus* (NRRL 3251), *Aspergillus carbonarius* (M333) and *Aspergillus parasiticus* (CBS 100926), and one yeast *Candida albicans* (27/4). Muller-Hinton agar was used to cultivate bacterial strains, whereas Sabouraud dextrose agar was used to cultivate fungus. Every microbial strain was cultured for 24 hours at 37 °C.

2.6.2 Disc diffusion assay

The disk diffusion assay published by [20] was used for assessing the antibacterial activity of *P. lentiscus* oil. The harvested microbial cultures were suspended in sterile saline (0.9% NaCl), with cell density adjusted to 0.5 McFarland for bacterial and yeast suspensions and 0.4 for fungus suspensions. In sterile Petri dishes, 20 mL of molten agar mixture was poured. A suspension of bacteria and fungi (100 µL) was distributed on the nutrient agar plates, and the plates were then aseptically dried for 2 hours at room temperature. Sterile paper disks (6 mm in diameter) were impregnated with 10 µL of oil and placed on the infected surface. Prior to incubation, the Petri plates were incubated at 4 °C for 1 to 2 hours to allow the oils included in the discs to diffuse. Finally, bacteria were cultured at 37°C for 18-24 hours, while yeast and fungi were incubated at 30°C for 48-72 hours. Measurement of the zone of inhibition was used to assess antimicrobial activity against the micro-organisms tested.

2.7 Statistical analysis

Statistical analysis was performed using SPSS Statistics 22 (SPSS Inc., Chicago, USA). All analysis was conducted in triplicate, and results were expressed as mean $\pm$ SD. Independent samples *t*-test was used to test the statistical differences between the means of different parameters obtained from the extraction methods HLCO and HLHO. Differences were considered statistically significant at a significance level of $p < 0.05$.

3. Results and Discussion

3.1 Extraction

In Algeria, the oil from the fruit, which is frequently obtained by traditional method, is utilized in conventional medicine as well as for a variety of dietary and other purposes [21].

Extraction yields expressed as a function of dry matter (% DM) are shown in Fig. 3. In the current study, we found that the extraction method affected the oil yields. Extraction by cold pressing method gave a yield three times higher ($15.3 \pm 0.23\%$) than the hot extraction method ($5.2 \pm 0.18\%$). Similar results were reported by [22], obtaining 6% with the traditional method. However, [23] obtained very high yields (16.66%) for oil traditionally extracted from fruit harvested in Bejaia. The cold-pressed oil harvested in Guelma (north-east Algeria) achieved a yield of 31%, which is higher than ours [24]. This yield was also high (22.71%) in Beja (north-west Tunisia) [25]. Contrary, [6] revealed that the oil yields obtained by cold pressing method from seeds collected from different regions of Tunisia, were different. It seems that the cold pressing method increases the yield compared with traditional method. At an industrial scale, vegetable seed oil is preferably

extracted by continuous screw pressing [26].

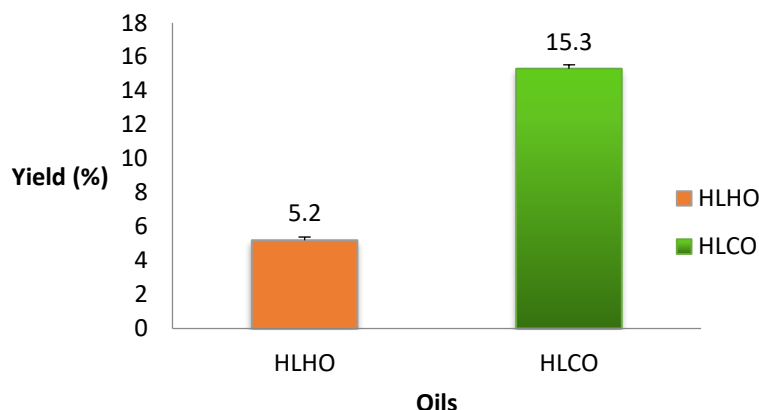


Fig. 3 Extraction yields (% DM) of Hodna *P. lentiscus* oils. Different letters symbolized significant differences

($P < 0.05$) by mean of the *t*-test. Data were represented as mean $\pm$ SD of three measurements.

3.2 Total phenolic content

The total phenolic content is an important factor to evaluate the quality of edible oils due to their contribution in flavor and protecting the fatty acids from oxidation [27]. The total phenolic contents of the two oils are presented in Fig. 4. The total phenolic content of HLCO (94.54 mg GAE/100g) was twice that of HLHO (48.02 mg GAE/100g), making HLCO a richer source of phenolic compounds. The significantly ($p < 0.0001$) lower total phenolic content of HLHO compared to HLCO might be attributed to its relatively higher water content resulting from the traditional extraction process involving water addition [28].

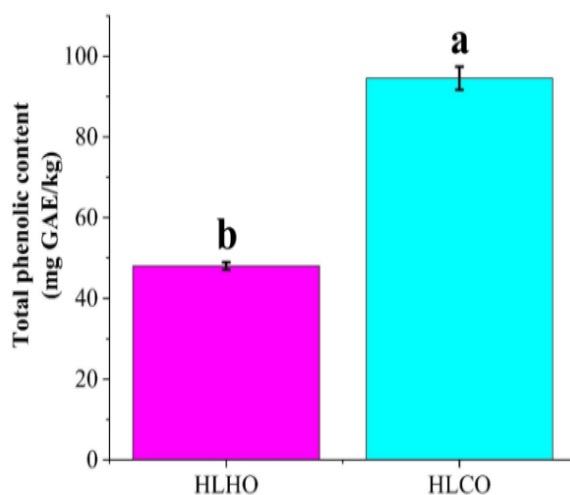


Fig. 4 Total phenolic content of Hodna *P. lentiscus* oils. Different letters symbolized significant differences ($P < 0.05$) by mean of the *t*-test. Data were represented as mean $\pm$ SD of three measurements.

Regarding HLHO, our finding was higher than that of [29], who reported a total phenolic content of 25.15 mg GAE/100 g oil for Algerian oil extracted with traditional method. HLCO's total phenolic content was higher than those of produced from the seeds grown in Tunisia (20.41 mg GAE/100g) and Cilento/Italy (43.50-61.60 mg GAE/100g), while lower than that of Moroccan pressed oil (22.61 mg GAE/g). The higher

total phenolic contents recorded in the current study, particularly HLCO, contribute to improved resistance to oxidation, highlighting that the sub-arid origin of our lentisk and the cold extraction process offer superior oil quality [30].

The literature shows a diversity in the content of phenolic compounds in lentisk oils. These variations are likely caused by a variety of variables, including genotypic factors, biotic circumstances (species, organ and physiological stage), abiotic (edaphic) conditions, soil type and bioclimatic stages [27]. Similarly, the growing season, growth stage and extraction technique may be involved [31]. Furthermore, as reported in the literature, numerous substances can react with the Folin-Ciocalteu reagent to produce a high apparent phenol content, meaning that the standard spectrophotometric determination of the polyphenols content with this reagent is not specific to polyphenols [32].

3.3 DPPH-radical scavenging activity

The DPPH-radical scavenging assay provides an estimation of the total contents of reducing agents and is commonly used for the determination of the antioxidant activity of various plant extracts [33]. Previously, the strong DPPH and ABTS radical scavenging activities as well as pancreatic lipase inhibitory activities of the fruit seed oils were confirmed by [5]. The anti-radical capacities of the oils are shown in Fig. 5. Both oils showed very high free radical scavenging activity, but that of HLHO (113.81 mM TE/100 g) was slightly ($p > 0.05$) higher than that of HLCO (107.47 mM TE/100 g). In the current study, the samples had greater antiradical activity than those of lentisk oils of overripe pulp (80 μg of Trolox/g oil), whole unripe fruit (28 μg of Trolox/g oil), and unripe seed (0.9 μg of Trolox/g oil) and the oils obtained from the seeds harvested in different regions of Tunisia by [34], [6]. [5] highlighted that seed oils exhibit potent DPPH and ABTS radical scavenging activity and pancreatic lipase inhibitory activity *in vitro*.

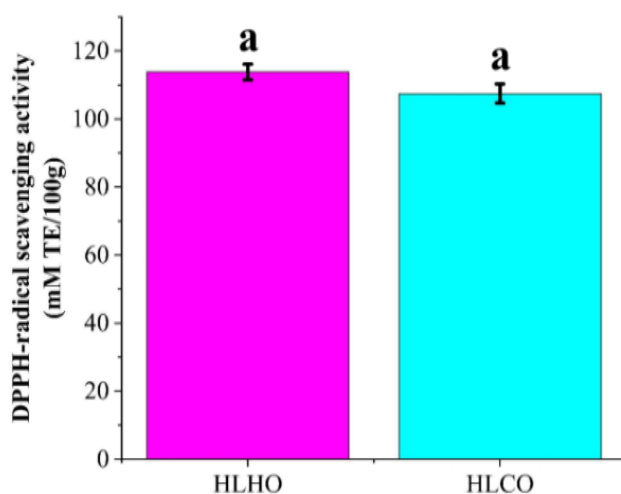


Fig. 5 DPPH-radical scavenging activity of Hodna *P. lentiscus* oils. Different letters symbolized significant differences ($P < 0.05$) by mean of the t-test. Data were represented as mean $\pm$ SD of three measurements.

The phytochemical composition of seed oils, which includes tocopherols, phenolic compounds, carotenoids, and chlorophyll, contributes to their antioxidant activity. Tocopherols and phenolics are the main antioxidants found in oils [27]. It is important to note that although having different phenolic compositions, they have comparable scavenging power. Lentisk seed oils exhibited significant *in vitro* antioxidant activity owing to the presence of inherent powerful antioxidants. The antioxidant activity of HLCO may be due to its high content of polyphenols. The type and position of hydroxyl and the type of phenolic compounds determine the

antioxidant capacity [35].

One of the most important chemical properties of tocopherols is their antioxidant activity, and it is believed that all their biological activities derive from their capacity to protect lipids from oxidation [36]. Consequently, the antioxidant activity of these oils, particularly HLHO, may also be due to their tocopherol isomers contents. Tocotrienols may also be at least partly involved in this activity. Tocopherols (vitamin E) along with phenolic compounds are effective natural radical scavengers and defend the body against free radical attack by protecting monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) from oxidation [37]. α -tocopherol and polyphenolic substances can act synergistically *in vivo* [38]. Usually, in fats and oils, α -tocopherol has better antioxidant activity than γ -tocopherol [39]. In addition to phenolics and tocopherols, various compounds, including phospholipids, and certain fatty acids, like linoleic acid may be responsible for the antioxidant potent of mastic oils [40], [41]. On the other side, many antioxidants, possibly certain pigments such as carotenoids, some-times leads to a reduction in the antioxidant activity of components like tocopherols and phenolic compounds [42].

3.4 Antimicrobial activity

Antimicrobial susceptibility testing can be applied to epidemiology, drug development, and treatment outcome prediction. Our results demonstrated that both oils did not have antimicrobial activity against five pathogenic bacteria (*S. aureus*, *B. cereus*, *L. monocytogenes*, *E. coli* and *P. aeruginosa*) and six fungi strains (*U. ramanniana*, *F. culmorum*, *A. flavus*, *A. carbonarius*, *A. parasiticus* and *C. albicans*). Our observations are in accordance with the findings of [43], who revealed that *P. lentiscus* seed oils collected from different regions of eastern Algeria (El Taref, Skikda and Guelma) did not have inhibitory effect against *B. klebsiella* and *S. aureus*, while demonstrating efficiency against three fungal strains, including *Verticillium sp.*, *Pythium sp.* and *Phytophthora sp.* Likewise, no antibacterial activity against *P. aeruginosa*, *K. pneumoniae* and *S. aureus* was detected in the oils obtained from seeds harvested from different regions from eastern of Algeria, but antifungal activity against *Phytophthora sp.*, *Verticillium sp.* and *Pythium sp.* was observed [21]. On the contrary, no inhibitory effect of Algerian lentisk oil against Gram-negative bacteria *E. coli* and *P. aeruginosa*, nor against fungi (*A. niger*, *A. flavus*, *M. rammaniarum*, *A. ochraceus*, *A. parasiticus*, and *C. albicans*) was detected. However, this oil exhibited only a small zone of inhibition (diameter: 1 mm) against the two Methicillin-resistant *S. aureus* strains [29]. In accordance with these, any inhibitory effect against *E. coli* and *S. typhimurium*, [39] *L. monocytogenes*, *E. coli*, *B. subtilis*, *S. typhimurium*, *S. Arizona* and *P. aeruginosa*, *A. flavus* and *C. albicans* was not reported for *P. lentiscus* fruit oils produced in Tunisia [10], [44]. On the other side, our findings contradict those of [45], who reported antimicrobial activity against *L. innocua*, *S. enterica*, *E. faecalis*, and *S. flexneri* and antifungal activity against *Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata* for lentisk fruit oil collected from the north of Tunisia (Bizerte).

Despite the richness of our oils in polyphenols, no inhibitory effect was observed against the microbial strains tested, although these compounds are known for their antimicrobial power [46-48]. Several other secondary metabolites also have potential antimicrobial properties [49].

The method used to assess antimicrobial activity can influence the results [50]. Consequently, comparison between the results of antimicrobial studies is often challenging due to the utilization of different, non-standardized approaches for inoculum preparation, inoculum size, growth medium, incubation conditions, and end-point determination. The standard technique for conducting regular anti-microbial susceptibility testing in many clinical microbiology laboratories is the agar disc diffusion test [51].

4. CONCLUSION

The present study reveals for the first time the phenolic content, antioxidant, antibacterial, and anti-fungal properties of Hodna sub-arid lentisk seed oils obtained through two different process. The sub-arid origin of *P. lentiscus* and the extraction method considerably influenced the polyphenols content of both oils. HLCO contained a greater concentration of phenolic compounds compared to HLHO. Despite HLHO and HLCO exhibited remarkable DPPH-radical scavenging abilities, their antibacterial and antifungal activities were comparatively weak. These oils may serve as potential natural sources of bioactive compounds that may have promising applications in the health, food, and cosmetics industries.

Acknowledgments

This study was supported by the General Direction of Scientific Research and Technological Development (DGRSDT), the Ministry of Higher Education and Scientific Research of Algeria (MESRS) and Manisa Celal Bayar University, Turkey.

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