Journal of Medicinal Plants for Economic Development
ISSN: (Online) 2616-4809, (Print) 2519-559X

In vitro anti-onychomycosis and radical-scavenging activities of *Pistacia lentiscus L.*

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Dates:
Received: 11 May 2025
Accepted: 18 June 2025
Published: 31 July 2025

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Background: Herbalists sell extracts of *Pistacia lentiscus*, prepared by small businesses to treat certain dermatological and digestive problems, without side effects. Very little research has discussed the main compounds and biological activities of the leaves of this species.

Aim: Phytochemical screening, quantify phenolic compounds, and evaluate the biological activities of *Pistacia lentiscus*. L.

Setting: The leaves are taken from the female plants of *P. lentiscus* L., which grow in the forest of Canastel, Oran, (Algeria).

Methods: A phytochemical screening was performed on *P. lentiscus. L* leaves and two extraction techniques were used for a comparative study. The total polyphenolic compounds, condensed tannins and flavonoids was quantified by the spectrophotometric methods. The antioxidant activity of the extracts was determined by two different techniques. The minimum inhibitory concentration of the extracts against *Fusarium sp* causing onychomycosis, was determined using the microdilution method.

Results: Phytochemical screening indicates the presence of flavonoids, gallic tannins, saponins, anthocyanins, cardiac glycosides, leucoanthocyanins and quinones. The hydroalcoholic extract contained the highest amounts of total phenols, flavonoids, and condensed tannins and the most potent antioxidant and anti-*Fusarim* activities.

Conclusion: Condensed tannins were found to be the most abundant phytochemical constituents. This suggests that the biological activities observed in this study may be largely attributed to the presence of condensed tannins.

Contribution: The results of this study justify the use of *P. lentiscus* extracts in phytotherapy, especially for treating dermatological (onychomycosis) and digestive diseases, and open new research perspectives to promote this species in different sectors.

Keywords: *Pistacia lentiscus. L*; phytochemical; polyphenols; antioxydatives; onychomycosis.

Introduction

Plants are an exceptional source of exogenous antioxidants. They are capable of synthesising and accumulating a wide variety of secondary metabolites that effectively prevent cellular oxidation. As a result, they contribute to protection against life-threatening diseases, including neurodegenerative and cardiovascular diseases, and inhibit tumour cell formation (Muscolo et al. 2024). Similarly, medicinal plant extracts exhibit very interesting antimicrobial activities because of their secondary metabolism molecules (Panda et al. 2025). Recently, several research works have been devoted to the phenolic compounds, and their applications in different industrial fields, including the pharmaceutical production sector and the food-processing sector because of their powerful biological activities, especially antioxidant and antimicrobial activities (Bolat et al. 2024; Li et al. 2024).

Pistacia lentiscus L. is an important component of scrubland, forests, and maquis in the Mediterranean region. It is called Mastic and in Algeria and Arab countries, 'Derou'. *Pistacia lentiscus* L. is a shrub belonging to the Anacardiaceae family. It is dioecious. Its leaves are always paripinnate and persist in winter. It has 2–5 pairs of leathery, dark green, lanceolate leaflets with a strong odour, and a narrowly winged petiole. It flowers from April to May, with dense, short,

How to cite this article: Belmokhtar, Z., Sebaa, H.S., Sadli, H.E.M., Mezemaze, R.e.h., Merad, Z., Bouiadjra, S.E.B. et al., 2025, '*In vitro* anti-onychomycosis and radical-scavenging activities of *Pistacia lentiscus L.*', *Journal of Medicinal Plants for Economic Development* 9(1), a290. https://doi.org/10.4102/jomped.v9i1.290

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spike-like clusters of inflorescences. The fruit is globose, red, then black (Quézel & Santa 1963).

The various parts of *P. lentiscus* have long been used in traditional medicine. It has been used to treat eczema, jaundice, skin infections, gastrointestinal disorders, throat infections, and kidney stones. Its oils are used as a healing agent and for the repair of wounds and burns (Elloumi et al. 2022). In Algeria, *P. lentiscus* is very useful for the treatment of digestive disorders, respiratory diseases, circulatory disorders, allergies, and skin problems (Boudieb, Ait Slimane- Ait Kaki & Amellal-Chibane 2019; Zitouni et al. 2023). The oil of *P. lentiscus* from Algeria, which is very useful in herbal medicine, contains a significant quantity of phenolic compounds and is very rich in fatty acids particularly oleic, palmitic, linoleic, palmitoleic and stearic acids (Belyagoubi-Benhammou et al. 2018).

Phytochemical, biological and pharmacological studies confirm that this species has very interesting activities such as anti-ulcer, hypocholesterolemic, hepatoprotective, anti-diabetic, anti-atherogenic, anti-cancer and anti-inflammatory (Sehaki et al. 2023). As part of the valorisation of species used in phytotherapy, we were interested in *P. lentiscus* from the Canastel forest: a species widely used by the local population as a medicinal plant, but the chemical composition of this species that grows in the Canastel forest and its biological activity have not yet been studied to date. This investigation allows for the first time to determine the phytochemical composition, the antioxidant and antifungal capacities of *P. lentiscus* from this station. In this study, we were interested in carrying out a comparative study between two techniques for extracting secondary metabolism molecules in order to determine the phytochemical composition of *P. lentiscus*, quantify phenolic compounds (total polyphenols, flavonoids, and condensed tannins), evaluate antioxidant and fungal activities, and determine the dominant class of molecules responsible for the biological activities of *P. lentiscus* L.

Research methods and design

Plant material

The leaves were collected at sunrise in October 2023 from female shrubs of *P. lentiscus* L., which grows in the Canastel forest, located in Oran (Algeria) on the edge of the Mediterranean Sea. The species was identified by Dr Asma El Zerey-Belaskri, specialist in biodiversity and plant taxonomy from Oran University, Ahmed Ben Bella 1. Algeria. The plant material was dried at room temperature and then crushed to a fine powder using a RETCH knife mill with a 0.75 mm filter, then stored in kraft paper bags.

Phytochemical screening

We began our study by conducting phytochemical tests on the powder of *P. lentiscus* leaves using standard procedures to identify the presence of secondary metabolites, including flavonoids, tannins, anthraquinones, saponosides, alkaloids, anthocyanins, leucoanthocyanins, cardiac glycosides, and quinones according to the methods of Harborne (1984).

Extraction method

We carried out two extraction techniques:

Extraction by boiling under reflux in water

Pistacia lentiscus leaves powder was mixed with distilled water at a percentage of 5%, then, the mixture was brought to a boil under reflux at 120 °C for 30 min. Then, the filtrate was evaporated using a rotary evaporator under vacuum at 50 °C. Finally, the extract obtained was stored at 4 °C until use.

Maceration in hydroethanolic solution (70%)

Pistacia lentiscus leaves powder (5 g) were extracted three times with 70% ethanol in a ratio of 1:10 (w/v) with stirring for 8 h at room temperature for each extraction. The three filtrates were collected and concentrated using a vacuum rotary evaporator at 50 °C, and then the solid crude extract was stored at 4 °C until use.

Quantification of phenolic compounds

Determination of total polyphenol content

Total polyphenolic compounds were quantified using the Singletonm and Rossi (1965) method. Exactly, 1 mL of Folin-Ciocalteu reagent diluted 10 times was added to 100 µL of extract at a concentration of 1 mg/mL. 300 µL of sodium carbonate (Na_2CO_3) at a concentration of 7.5% was added. The final volume was adjusted to 3 mL and then vortexed. The reaction mixture was left to stand for 90 nm in the dark. The absorbance reading was performed by spectrophotometer at a wavelength of 760 nm. A standard range was prepared using gallic acid from 0 to 1 mg/mL. The quantity of total phenolic compounds was expressed in mg gallic acid equivalents per g of dry extract (mg GAE/g extract).

Determination of total flavonoid content

Flavonoids were measured using the method of Dewanto et al. (2002). A volume of 2.5 mL of distilled water was added to 0.5 mL of extract at a concentration of 1 mg/mL, then 150 µL of sodium nitrite (5% $NaNO_2$) was added to the mixture before vortexing. Then, 150 µL of 10% aluminum chloride and 1 mL of 1 mol/L NaOH were added. The mixture was finally vortexed again and left to stand for 15 min in the dark. The absorbance reading was performed by a spectrophotometer at a wavelength of 510 nm. A standard range was prepared by catechin from 0 to 1 mg/mL. The results are expressed in mg equivalent to catechin per g of dry extract (mg EC/g extract).

Determination of condensed tannin content

Condensed tannins were quantified using the method of Burns (1971). A 5 mL of vanillin-HCl reagent was added to 100 µL of extract at a concentration of 1 mg/mL, then vortexed. After standing for 20 min in the dark, absorbance values were read at a wavelength of 500 nm using a spectrophotometer. A calibration range was prepared using catechin at a concentration ranging from 0 to 1 mg/mL.

Results are expressed as mg of catechin equivalent per gram of dry extract.

Study of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay

The ability of the extracts to scavenge free radicals was evaluated according to the protocol of Brand-Williams, Cuveliem and Bersetm (1995). A volume of 2.9 mL of DPPH solution, freshly prepared at a concentration of 60 µM/L, is added to 100 µL of different concentrations (from 1 to 0.0312 mg/mL) of the extracts to be analysed. After the mixture was vortexed and then left to rest for 30 min in the dark, absorbance readings were taken at a wavelength of 517 nm using a spectrophotometer. Ascorbic acid was used as a control. The percentage inhibition for each concentration was calculated using equation 1 below:

$$\% \text{ inhibition} = (A0\text{-}A1/A0) \times 100\% \quad \text{[Eqn 1]}$$

where: Al = Absorbance of the extract or standard

A0 = Absorbance of the control.

The results obtained were used to establish a linear regression between the percentage inhibition and the different concentrations. This linear regression allowed us to calculate the IC_{50}, which corresponds to the concentration of the extracts that eliminates 50% of the DPPH.

Total antioxidant capacity assay

The total antioxidant capacity of the extracts was measured according to the method of Prieto, Pineda and Aguilar (1999). Exactly, 2.7 mL of the reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) is added to 300 µL of extract at a concentration of 1 mg/mL. The mixture is vortexed and then incubated in a water bath at 95 °C for 150 min. After cooling to room temperature, the absorbance readings were made at 695 nm. A standard range was created using ascorbic acid at different concentrations (from 0 to 0.250 mg/mL). The results were expressed in milligram (mg) equivalent to ascorbic acid per gram of dry extract (mg EAA/g).

Anti-onychomycosis activity

The antifungal activity of the two extracts was carried out on *Fusarium* sp. taken from the nails of a patient suffering from onychomycosis, the fungus was isolated and identified by Professor Merad, Y, specialist in mycology and parasitology, head of the central laboratory of the University Hospital Abdelkader Hassani, Sidi-Bel-Abbes, Algeria.

The minimum inhibitory concentration (MIC) was determined in sterile 96-well microplates using the broth microdilution method, the technique described previously by Balouiri, Sadikim and Ibnsoudam (2016) with some modifications. In sterile 96-well microplates, the broth microdilution method was employed to evaluate the MIC. Under sterile conditions, suspend the *Fusarium* culture in 5 mL of 0.85% NaCl solution, then adjust the turbidity of the suspension according to the McFarland 0.5 scale; this turbidity corresponds to a fungal concentration of 1.5×10^6 (CFU/mL). A two-fold dilution series of the extracts was carried out in the wells using liquid Saboraud culture medium, to have a final volume of 50 µL in each well and a series of concentrations of the extracts from 125 mg/mL to 0.06 mg/mL. Finally, each well was aseptically inoculated with 50 µL of the *Fusarium* suspension whose turbidity had been adjusted. The results were read visually after 5 days of incubation at 28 °C (Belmokhtar et al. 2020). The MIC is defined as the lowest concentration of the extract where there is no observable growth of the *Fusarium* after incubation.

Statistical analysis

All measurements were performed in triplicate, in three separate experiments; results are represented as mean with standard deviation.

Ethical considerations

An ethical waiver to conduct this study was obtained from the Ministry of Higher Education and Scientific Research, University of Science and Technology of Oran Mohamed Boudiaf, Faculty of Natural and Life Sciences, Director of the Laboratory dated 11 May 2025.

Results

Phytochemical screening

The results of the phytochemical screening of *P. lentiscus L. leaves* are summarised in Table 1. Phytochemical tests show the presence of flavonoids, gallic tannins, saponins, anthocyanins, quinones, leucoanthocyanins and cardiac glycosides, and the absence of alkaloids and anthraquinones.

The presence of flavonoids, tannins, anthocyanins, and the absence of alkaloids were also determined in the leaves (Boudieb et al. 2019) and fruits of *P. lentiscus* (Belhachat et al. 2017) from other stations in Algeria. Secondary metabolism molecules found by phytochemical screening in *P. lentiscus L.* leaves are known for their beneficial biological activities and are useful in several industrial fields, in food, nutrition, health, cosmetics, agronomy (Elshafie, Camele & Mohamed 2023).

TABLE 1: Results of phytochemical screening of *Pistacia lentiscus* leaves from the Kanastel station (Oran).

Molecules	Pistacia lentiscus leaves
Flavonoids (cyanidin test)	+
Tannins	+ Tanins gallic
Quinones	+
Saponosides	+
Cardiac glycosides	+
Anthocyanins	+
Leucoanthocyanins	+
Alkaloids	Reaction Wagner – Reaction Mayer –
Anthraquinones	–

+, positive test; –, negative test.

Discussion

Quantification of phenolic compounds

The results of the quantification of the total polyphenol, flavonoids, and condensed tannin content of *P. lentiscus* leaf extract are summarised in Table 2.

The yields of polyphenols, tannins and flavonoids are slightly higher in the extract obtained by maceration in ethanol 70% compared to the extract obtained by decoction in water (Figure 1).

The significant quantity of phenolic compounds in alcoholic extracts compared to aqueous extracts of *P. lentiscus* leaves was also reported in the work of Bouriche et al. (2016).

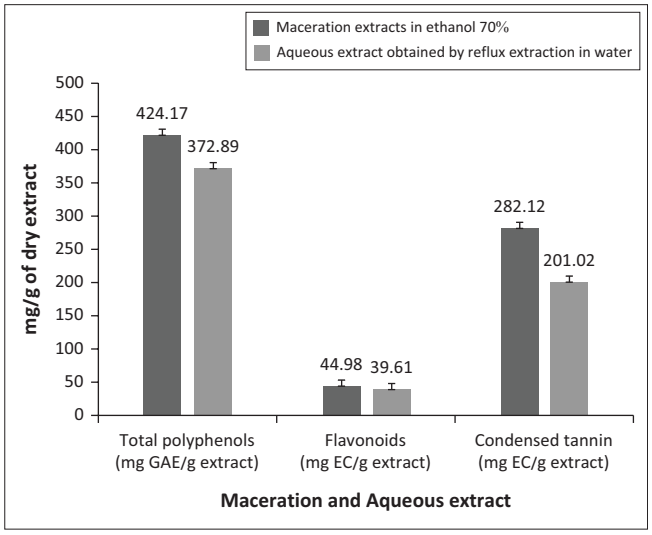
In comparison with other results carried out by other researchers on the same species collected from different stations, we observe variability in the contents of total polyphenols, tannins and flavonoids; some found contents almost similar to our results (Hemma et al. 2018), others found larger quantities (Belhachat et al. 2017) in particular total polyphenols, and others found much lower quantities (Mazari et al. 2022).

These results indicate that the extraction of phenolic compounds from *P. lentiscus* is influenced by the extraction method chosen, the polarity of the solvent, the duration and the temperature used for the extraction.

TABLE 2: Total phenolic, flavonoids, and condensed tannins contents of ethanolic 70% and reflux extracts of *Pistacia lentiscus* leaves.

Extracts	Total polyphenols (*mg GAE/g* extract)	Flavonoids (*mg EC/g* extract)	Condensed tannin (*mg EC/g* extract)
Maceration extracts in ethanol 70%	424.17 ± 06.89	44.98 ± 4.81	282.12 ± 08.26
Reflux extraction in water	372.89 ± 05.63	39.61 ± 3.87	201.02 ± 03.40

GAE, gallic acid equivalents; EC, equivalent to catechin.



GAE, gallic acid equivalents; EC, equivalent to catechin.

FIGURE 1: Total phenolics, flavonoids, and condensed tannins contents in maceration extracts in ethanol 70% and aqueous extract obtained by reflux extraction in water from *Pistacia lentiscus* L leaves.

Hadini et al. (2022) shows that altitude strongly affects the biochemical parameters of P. lentiscus leaves, particularly polyphenols. The seasonal variation of phenolic compounds in P. lentiscus leaves were significantly affected by the month of harvesting (Gori et al. 2020). Air quality plays an important role in the content of phenolic compounds, particularly flavonoids, which allows them to be used as biomarkers of air pollution (Belmokhtar et al. 2019).

The variation in the quantities of phenolic compounds in a plant extract is influenced by the chemical nature of the compounds, as well as by the method of analysis, the choice of standards and the presence of interfering substances (Do et al. 2014).

A very high quantity of tannins compared to the quantity of flavonoids obtained by the two extraction techniques indicates that tannins are the most dominant polyphenolic compounds.

The very high content of tannins in the leaves explains the astringent and bitter taste of the leaves of *P. lentiscus*, which is the subject of this study. Astringency arises from the precipitation of glycoproteins from the mucous secretions of the salivary glands resulting from strong interactions between tannins and proline-rich salivary proteins (Soares et al. 2018).

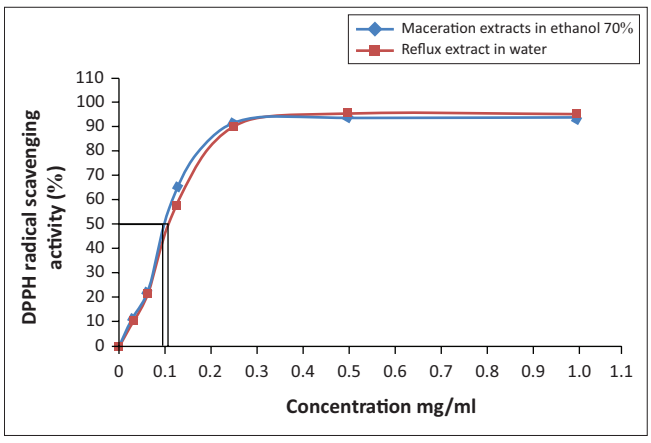
Study of antioxidant activity

Both techniques of analysis of the antioxidative activity show that the extract obtained by maceration in 70% ethanol has a slightly higher antioxidant activity than the extract obtained by boiling under reflux in water (Table 3 and Figure 2).

TABLE 3: Antioxidant activity (IC_{50}) on DPPH radicals and total antioxidant capacity of ethanolic 70% and reflux extracts of *Pistacia lentiscus* leaves.

Extracts	IC_{50} (DPPH) (*mg/mL*)	Total antioxidant activity Phosphomolybdate assay, (*µg/mg* EAA)
Maceration extracts in ethanol 70%	0.095 ± 0.002	212.33 ± 3.78
Reflux extraction in water	0.11 ± 0.004	196.25 ± 4.85

IC_{50}, Inhibitory Concentration at 50%; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EAA, equivalent to ascorbic acid.



DPPH, 2,2-diphenyl-1-picrylhydrazyl.

FIGURE 2: DPPH free scavenging activity of reflux extract in water and maceration extract in ethanol 70% of *Pistacia lentiscus* leaves.

The lowest IC_{50} (0.095 mg/mL) is that of the extract obtained by maceration in 70% ethanol at room temperature with an inhibition percentage of 91.77% at a concentration of 0.250 mg/mL; therefore, it has a slightly more powerful free radical scavenging activity compared to the extract obtained by boiling under reflux, which has an IC_{50} of 0.11 mg/mL with an inhibition percentage of 90.3% at 0.250 mg/mL (Figure 2). Similarly, the hydro-ethanolic extract has the greatest total antioxidant capacity (212.33 µg/mg) compared to the aqueous extract (196.25 µg/mg). The two extraction techniques used in this work prove to be more effective compared to the methanolic extract of the leaves of *P. lentiscus* collected from the Chréa forest (Algeria), which gives an IC_{50} = 0.121 mg/mL with a percentage of inhibition of 92.61% and a concentration of 0.4 mg/mL (Hemma et al. 2018).

Correlation analyses between antioxidant activity and the content of phenolic compounds carried out by Hadini et al. (2022) indicated that the antioxidant activity (DPPH) is correlated with the content of total polyphenols while the total antioxidant activity is correlated to the flavonoid content.

In our study and with the quantity of tannins obtained, it is suggested that tannins take part in this antiradical activity. The antioxidant properties of tannins are widely used in the food and medical fields. Tannins have attracted much attention from many researchers to elucidate the mechanism of action as antioxidant molecules because of their ability to prevent cardiovascular diseases, cancer and osteoporosis Tong et al. (2022). Other research shows powerful effects of hydrolysable tannins on the *in vitro* scavenging of free radicals evidenced, by the (DPPH) test, superoxide anions, $OH^{\cdot}$ and $OOH^{\cdot}$ Sieniawska (2015).

The results of Soldado, Bessa and Jerónimo (2021) show that the inclusion of plants or plant extracts rich in tannin in the diet of ruminants can improve the antioxidant status of animals by reducing oxidation reactions and protecting other antioxidant compounds. Phenolic compounds, and in particular tannins, in addition to their antioxidant activity, also allow the protection of the cell against deoxyribonucleic acid (DNA) damage Salar, Purewal and Sandhu (2017).

Total antioxidant activity assays are based on the reduction of Mo (VI) phosphate to Mo (V) phosphate by the sample, followed by the formation of a blue-green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is commonly used in laboratories to assess the total antioxidant capacity of plant extracts. The phosphomolybdenum assay involves the thermal generation of autoxidation during a prolonged incubation period at elevated temperature, thus representing a unique feature among *in vitro* antioxidant assays (Prieto et al. 1999). Comparing the extraction techniques in this study, 70% ethanol has the advantage of extracting polar and non-polar biomolecules.

Anti-onychomycosis

The results of the *in vitro* anti-onychomycosis activity of the two *P. lentiscus* extracts against *Fusarium* sp. are summarised in Table 4 and Photo 1.

Another study indicates that the minimum inhibitory concentrations of aqueous leaf extracts of *P. lentiscus* against nine bacterial strains range from 250 to 15.6 mg/mL. The same authors indicate that *Fusarium* sp. is more resistant to aqueous extracts of *P. lentiscus* than other species of the genus *Penicillium* (Debbabi, Nemri & Riahi 2017).

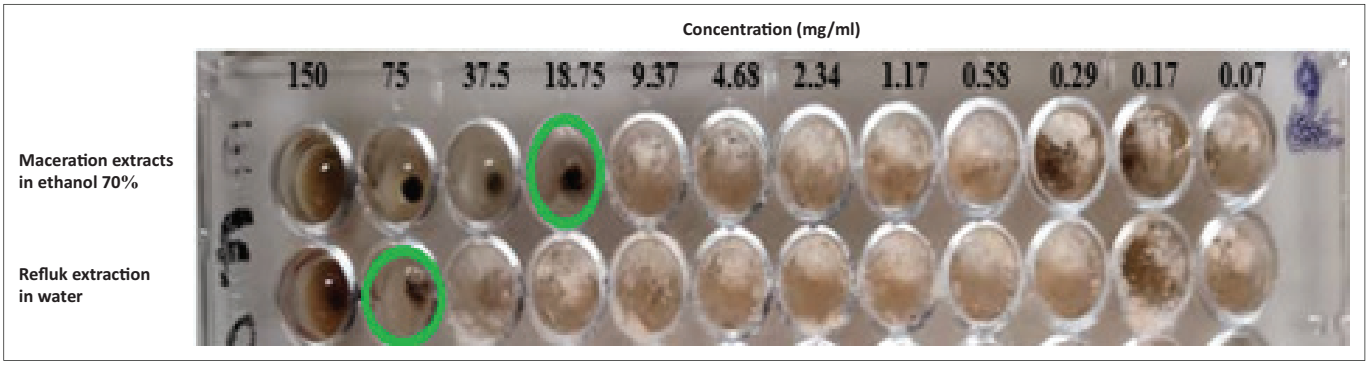
The various extracts of the aerial part of *P. lentiscus* show minimum inhibitory concentrations of around 50 mg/mL against *Escherichia coli* and *Staphylococcus aureus* (Missoun et al., 2017).

The results of the quantification of phenolic compounds show that condensed tannins are the most dominant molecules in the extracts of *P. lentiscus* leaves, which are the subject of this study. Several studies indicate that tannins have very powerful antimicrobial activity (Brighenti et al. 2021; Huang et al. 2024).

TABLE 4: Minimal inhibitory concentrations CMI (mg/mL) of *Pistacia lentiscus* leaf extracts against *Fusarium sp.*

Extracts	CMI (mg/mL) *Fusarium sp*
Maceration extracts in 70% ethanol	15.62
Reflux extraction in water	62.5

CMI, Minimal inhibitory concentrations.



Source: Photograph taken by Dr Belmokhtar Zoubir on 07 April 2025 in Sidi Bel Abbes, Algeria

Note: Photograph taken 5 days after inoculation, carried out in the central laboratory of the hospital.

PHOTO 1: Microplate bioassay showing the minimum inhibitory concentrations (MIC) of two extracts of *Pistacia lentiscus* leaves against *Fusarium* sp taken from the nail of a patient with onychomycosis.

Depending on the molecular structure of the different phenolic classes, their antimicrobial activity can occur through various mechanisms, reducing cellular efficiency and leading to bacterial death through their direct action on the DNA and ribonucleic acid (RNA) of microorganisms, thus reducing the expression of certain genes necessary for their multiplication, adenosine triphosphate production (ATP), and the chelation of essential metals in cellular metabolic pathways (De Rossi et al. 2025).

The antifungal activity of polyphenols can also occur through different mechanisms, including blocking the efflux pump, thus affecting the cell membrane, interfering with ergosterol synthesis, damaging the cell wall, or generating reactive oxygen species that can destroy fungal mitochondria (Davidova, Galabov & Satchanska 2024).

Conclusion

In Algeria, *P. lentiscus* leaves are widely used in phytotherapy but very little work has been performed on the phytochemical composition and biological activities of this species, and no previous work on *P. lentiscus* from the Canastel forest, Oran (Algeria). This study highlights the potential of *P. lentiscus* as a precious source of bioactive compounds, thus justifying its phytotherapeutic applications. Its potent antioxidant and antifungal activities are attributed to its richness in diverse classes of secondary metabolites. The study also concludes that astringent sensation of the leaves is because of the very large quantity of condensed tannins. Maceration in 70% ethanol at room temperature makes it possible to obtain a better yield of different phenolic compounds and to the most powerful antioxidant and anti-*Fusarium* activities. Quantitative analysis of phenolic compounds shows that condensed tannins are the most dominant molecules, this suggests that the biological activities may be because of condensed tannins. However, it is important to observe that synergistic or additive effects from other bioactive compounds in the extract may also contribute to the overall activity. Extracts from *P. lentiscus* leaves can be promising ecological alternatives to synthetic materials thanks to their exceptional biological properties. The extracts find many biotechnological applications such as biological additives, either as natural antioxidants, or as antimicrobials, in food, cosmetics and pharmaceutical industries. Finally, the results of this preliminary study undoubtedly encourage the scientific community to deepen this research through qualitative analyses of phenolic compounds, in particular condensed tannins, and to study other biological activities, in particular antimicrobial activities, to specify other microbial strains sensitive to extracts of this species, which allows artisanal manufacturers of *P. lentiscus* extracts to improve their production by formulating new, more effective and more precise healing products.

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

Z.B. contributed towards the conceptualisation, methodology and writing of the original draft. H.S.S. assisted with the methodology. H.E.M.S. assisted with the format analysis. R.e.h.M. assisted with the software. Z.M. assisted with the investigation. S.E.B.B. assisted with the resources. Y.M. assisted with the supervision.

Funding information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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