Ethnobotanical Survey, Phytochemical Screening and Antioxidant Activity of Methanolic Extracts of *Pistacia lentiscus L.* Growing in Northwestern Algeria

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Keywords: *antioxidant activity, ethnobotanical study, methanolic extract, phytochemical screening, Pistacia lentiscus L.*

In the context of biological studies on an antidiabetic plant, we conducted an ethnobotanical study of *Pistacia lentiscus L.,* collected from two regions in the Northwest of Algeria: Mesra (Mostaganem city) and Mohammadia (Mascara city), followed by a chemical and antioxidant studies of methanolic extracts the leaves of this plant. Ethnobotanically, the plant has a large use, especially in traditional medicine as antidiabetic, anti-inflammatory, antibacterial and cosmetics, such as polishing teeth and maintaining gums, moisturizing hair and protecting follicles, polishing skin and perfuming. Also, results showed its richness in active metabolites, such as polyphenols, flavonoids and tanins. The Mesra methanolic extract was more effective than Mohammadia's one with 90.12 ± 2.74 mg EqGAc/g DW, 41.86 ± 1.52 mg EqCer/g DW, 27.45 ± 056 mg EqCat/g DW while Mohammadia extract revealed 80.31 ± 1.42 mg EqGAc/g DW, 33.92 ± 1.71 mg Eq Cer/g DW, 27.61 ±1.53 mg EqCat/g DW for phenolic compounds, flavonoids and tannins respectively. In addition, the antioxidant study revealed a powerful antioxidant effect with an IC_{50} of 0.06 mg/mL and 0.1 mg/mL for methanolic extract. This antidiabetic plant is valuable from a health point of view, so we are seeking to confirm another biological activity *in vitro* and *in vivo.*

Introduction

Faced with the inefficacy of chemical medications and their impact on human health, especially in people with chronic diseases, scientists have focused on using medicinal plants by referring to ancestral knowledge and traditional pharmacopoeia for the treatment of chronic diseases such as diabetes which requires long-term treatment [1-5]. *Pistacia lentiscus L.,* called "Darw", a tree of about 3 meters [6], is widespread in the north of Africa, especially in Algeria and Morocco [7, 8]. This plant is a traditional medicinal plant; used for a long time and has been classified as antidiabetic, anti-inflammatory, antimicrobial, antioxidant, anti-ulcer, anti-cancer and antitoxin in general [9-14]**.** Fruits of *Pistacia lentiscus* give an edible oil, which is rich in unsaturated fatty acids such

as oleic and linoleic [15, 16]. In Algeria, the fruit's oil is used by the population in traditional medicine in many ways, as an antidiarrheal and a constituent of cattle feed. However, it is not only a traditional remedy and an aromatic plant, but also a powerful herbal product with various biological properties. For this, we have chosen this plant in order to achieve at first, an ethnobotanical study of *P. lentiscus L.*, collected in two regions in the Northwest of Algeria (Mesra municipality of Mostaganem) and (Mohammadia municipality of Mascara), then a comparative chemical study between the two varieties (phytochemical screening and antioxidant activity of their methanolic extract).

Experimental part

Ethnobotanical study

This statistical study on *Pistacia lentiscus L.* (Anacardiaceae) [17] were conducted on three categories of persons: phytotherapists, herbalists and traditional healers in the Northwest of Algeria Using the questionnaire sheets, ethnobotanical field surveys were conducted for 3 months. The approach of the categories of interviewees was based on dialog in local language about:

- Previous plant information.
- Professional option of field experts.
- Age and sex of therapists.
- Rate of health use of the plant (therapeutic, cosmetic, preventive or food taste).
- Used part of the plant (leaves, fruits, aerial part or resins).
- Usable state of the plant (dried or fresh).
- Plant harvest seasons.
- Instructions for use (herbal tea, powder, essential oils, fatty oils or extracts).
- Method of administration (oral, massage, rinsing or brushing).
- The usual practice (infusion, decoction, inhalation or external use).
- Diseases treated after oral administration.
- Diseases treated after administration through the respiratory tract.
- Diseases treated after administration by external use.
- Cosmetic uses of the plant.

Phytochemical study

Plant material

The aerial parts of plant material (leaves) of *Pistacia lentiscus L.* (Anacardiaceae) [17] **(Figure 1).** were collected from two regions of western Algeria, Mesra (municipality of Mostaganem) and Mohammadia (municipality of Mascara) **(Figure 2)** in 2022. This plant was identified by *a botanist* in the Department of Biology at Mascara University. A referenced specimen, *AN00001,* was introduced in our university's WAMAP-base of the Laboratory of Bioconversion, Microbiological Engineering and Health Safety (LBGMSS).

Figure 1. *Pistacia lentiscus L.* (Darw) collected in Mesra. Mostaganem (Algeria).

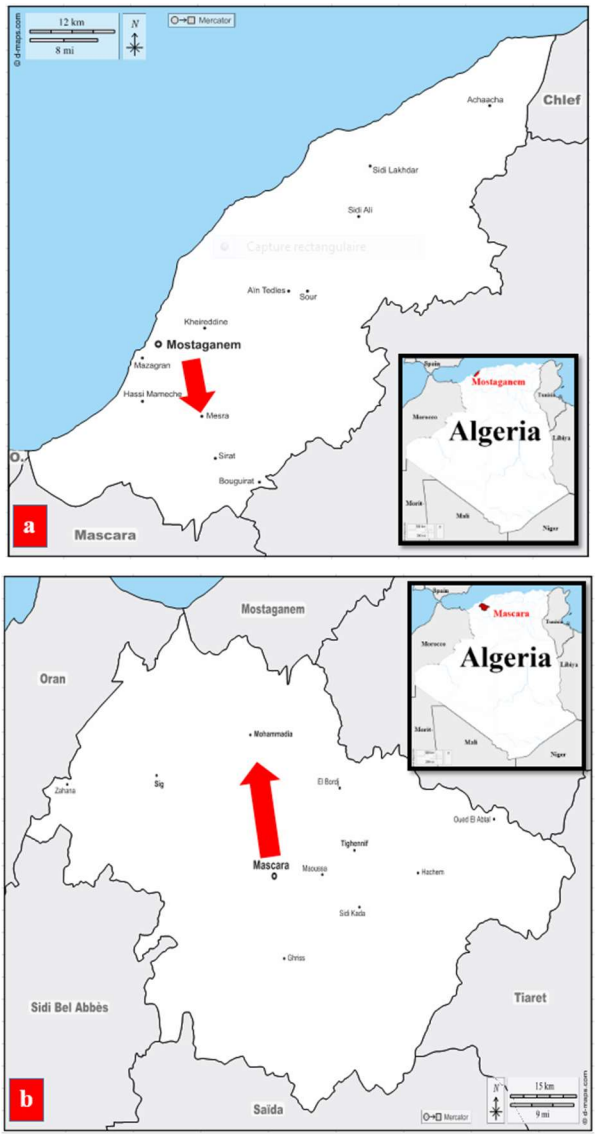

Figure 2. The geographical location of the area of *Pistacia lentiscus L.* (d-maps.com, 2022)

Preparation of the methanolic extract of Pistacia lentiscus

Dried leaves were crushed using an electric grinder. The obtained powder was stored in glass boxes at room temperature and protected from light and humidity. The methanolic extract was prepared by the cold maceration method [18] by placing 25 g of *P. lentiscus L.* powder from each varieties in 250 mL of methanol under stirring conditions for 24 hours at room temperature, followed by filtration using Wattman paper. The solvent was evaporated and then lyophilized to obtain powder then stored in a shaded glass container until use [19].

Determination of yield

The yield was expressed as a percentage according to the following formula: $Y\ (\%) = (m_{ex} / m_p)*100$. [20]. m_{ex}: mass in grams of the resulting dry extract. m_p: dry mass in grams of vegetable powder to be treated.

Qualitative phytochemical analysis (Phytochemical screening)

Phytochemical screening is a qualitative study aimed at researching the main chemical groups (tannins, alkaloids, flavonoids, free anthraquinones, anthocyans, triterpenes, saponins, terpenoids, steroids, alkaloids and reducing compounds) contained in the plant extract. Precipitation and complexation reactions with the formation of insoluble and coloured complexes are essential tests for characterization [21-23].

Quantitative phytochemical analyses

These tests were carried out for methanolic extracts of the two varieties of *P. lentiscus L*. The assay was carried out on three essential and active organic molecules: total polyphenols, flavonoids and condensed tannins.

Determination of phenolic amount

The quantification of total polyphenols is carried out by a method based on Folin Ciocalteu [24]. They used an estimated concentration of 1 mg/mL of extracts, the initial mixture prepared by combining 0.1 ml of the extract to be analyzed and 2 ml of 2% sodium carbonate solution. Shake the whole and incubate at room temperature for 5 minutes. 0.1 mL of 0.2N Folin Ciocalteu reagent was added and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 760 nm to plot the calibration curve using gallic acid at different final concentrations. The contents of total phenols in the extracts were expressed in milligrams (mg) equivalent of gallic acid per gram (g) of the weight of the dry weight (mg EqGAc / g DW) [25].

Determination of total flavonoids

The total flavonoids are quantified by the colorimetric method with aluminum trichloride and sodium hydroxide [26]. Using an estimated concentration of extracts (1 mg /1 mL). The initial mixture was prepared by combining 0.5 mL of the extract to be analysed, and 2 mL of distilled water in three stations, starting by adding 150 µL of the 15% sodium nitrite solution. After incubation at room temperature for 6 minutes, 150 µL of 10% aluminum chloride was added and incubated at the same conditions. Then, the operation was completed with 2 mL of 4% sodium hydroxide and adjusted the total volume to 5 mL with distilled water. The final mixture was stirred and incubated at room temperature for 15 minutes. Finally, the absorbance was measured at 510 nm. A standard range was produced in parallel under the same operating conditions using quercetin as a positive control at different final concentrations [27-29].

Dosage of condensed tannins

The vanillin method uses the quantification of tannins in an acidic medium [30]. Using an estimated concentration of extracts (1 mg/1 mL). The initial mixture was prepared by combining 50 µL of the extract to be analyzed and 1.5 mL of the vanillin/methanol solution using a vortex. Then, 750 µL of concentrated hydrochloric acid was added and incubated for 20 minutes at room temperature. The absorbance was measured at 550 nm. The amount of tannins was estimated in milligrams (mg) of catechin equivalents per gram (g) of dry extract weight (mg EqCat / g DW) from the calibration curve [31].

Antioxidant activity of Pistacia lentiscus L. methanolic extract

The antioxidation power was selected using DPPH (2,2-diphenyl-1-picrylhyrazyl) of violet colour, which was reduced to yellow-coloured 2,2 diphenyl-1-picryl hydrazine

[32,33]. DPPH is a stable free radical that absorbs at 517 nm to determine the percentage of inhibition proportional to the sample's anti-radical power [34]. For this, the concentration 40 mg/mL of DPPH and the different concentrations (10, 5, 2.5, 1.25, 0.62, 0.31 and 0.16) mg/ml of the methanolic extract *P. lentiscus L.* were prepared. 50 µL of extract and 1950 µL of DPPH were added. Also, the white tube was prepared by adding 50 µL of DPPH. The whole was incubated for 30 min at room temperature in the dark. Absorbance (λ = 517nm) was measured. The formula below determines the percentage inhibition of DPPH based on the average of three measurements obtained for the extract. For the additional statistical and comparative study, we performed the same experiment using ascorbic acid (Vitamin C) instead of the extract [35,36]. I%=((Ac-At) /Ac)*100. I %: percentage inhibition of DPPH. Ac: absorbance of the negative control. At: absorbance of the tested extract.

Determination of the 50% inhibitory concentration of radicals (IC50)

The 50% inhibitory concentration (IC_{50} or EC_{50} for Efficient Concentration 50%) was calculated graphically by linear or logarithmic regressions of the inhibition percentages according to different concentrations of the methanolic extract tested [37].

Statistical study

All experiments were made in duplicate. All data are presented as means ± SD. Total polyphenols, flavonoids and tannins were examined for statistical significance using analysis of variance (Excel, ANOVA two-factor test with repetition and the Post hoc test, which was assured by IBM SPSS statistic version 25). A p-value of 0.05 was considered the threshold to declare a statistically significant difference.

Results and discussion

Ethnobotanical study

According to the results of this study, it was found that 92% of the therapists know very well *Pistacia lentiscus L.* **(Figure 3)**; also, they were divided into three categories, 42% were traditional healers, while 22% were a phytotherapist doctors **(Figure 4).**

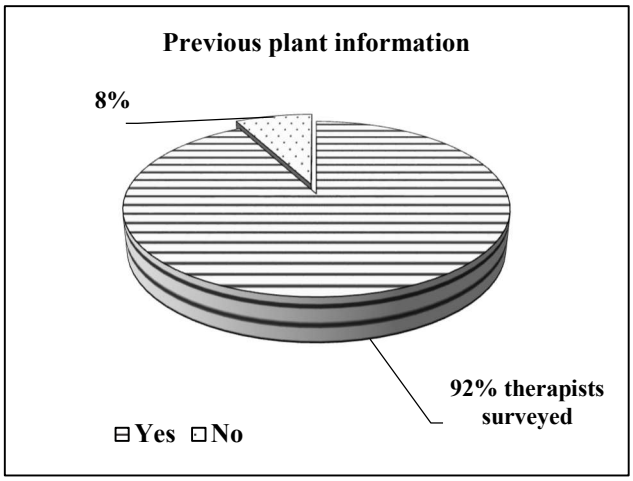

Figure 3. Previous plant information in therapists.

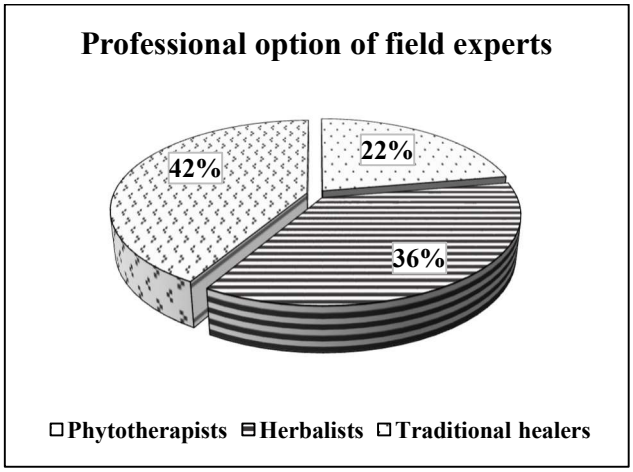

Figure 4. Professional categories of field experts.

Also, it was found that men presented the main category treating by *P. lentiscus L.* medicinal plant (64%) **(Figure 6)**. **Figure 5**, revealed that most of therapists were older.31% were between 45 and 60 years old, and 47 % were over 60 years old.

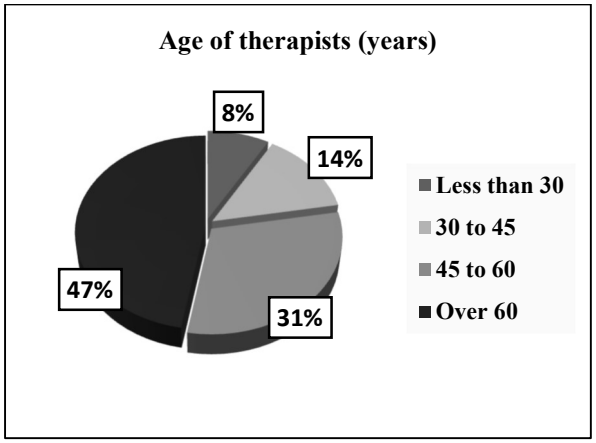

Figure 5. Different age intervals of surveyed therapists.

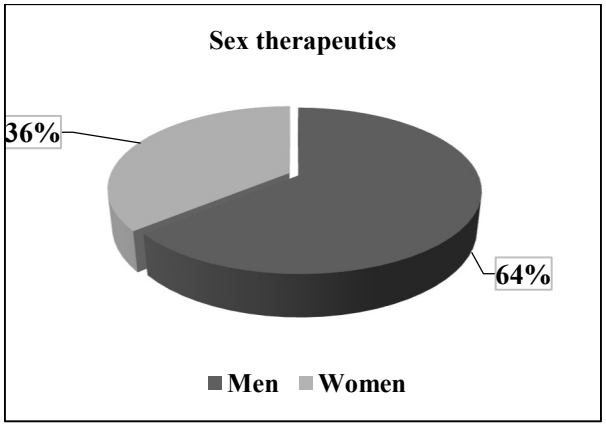

Figure 6. Sex of surveyed therapists.

The methods of preparing plant for treatment and the methods of their use have been graphically represented, results shown 48% of primary data collected from therapists indicated that the *Pistacia lentiscus L.* was primarily therapeutic **(Figure 7)**; its use in this field has been confirmed by many researchers [12, 16, 38-40].

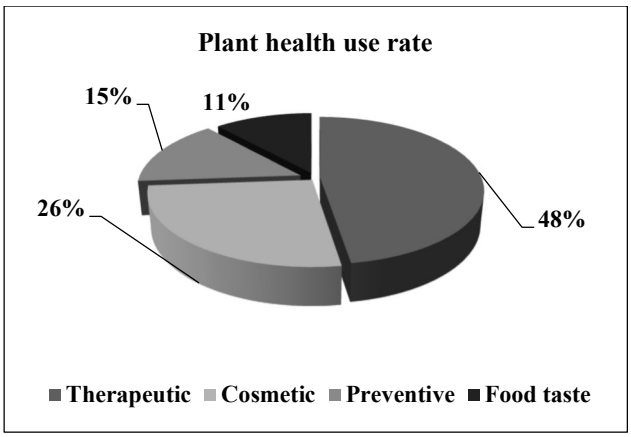

Figure 7. Sanitary use rate of *Pistacia lentiscus L.*

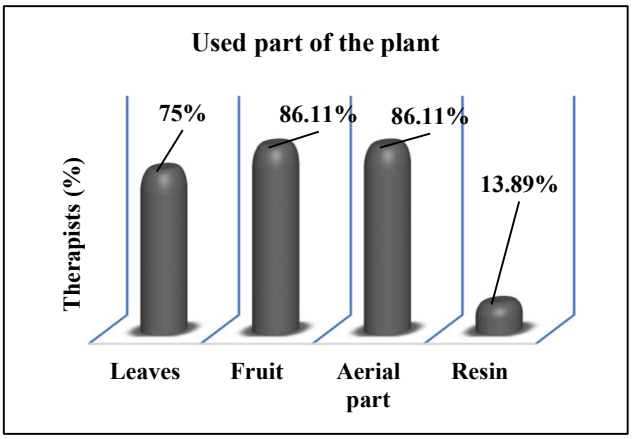

Figure 8. Percentage of use of parts of the plant.

When 86.11% of therapists agreed that the aerial part and fruits were the most effective, 75% confirmed that the leaves are the most critical part of the plant **(Figure 8)**, Previous studies also confirmed the use of resin in treatment [41]. This is due to the large stock of compounds with biological activities, as well as secondary metabolites**,** in addition to the possibility of the speed of harvest and ease of collection [42]. 70% of data collected from individuals treating patients with the plant prove their use of the plant after drying **(Figure 9)**.

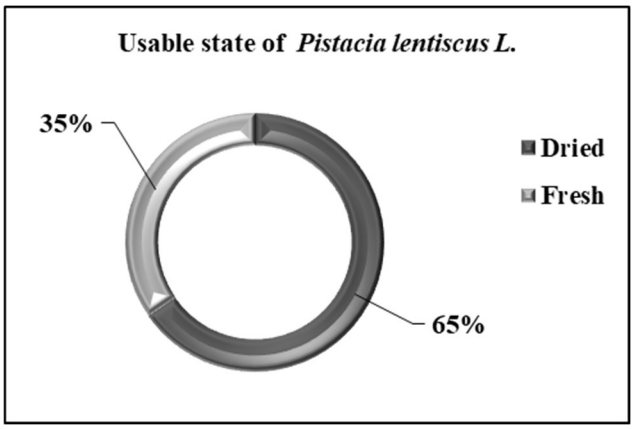

Figure 9. Use rate according to the state of the plant.

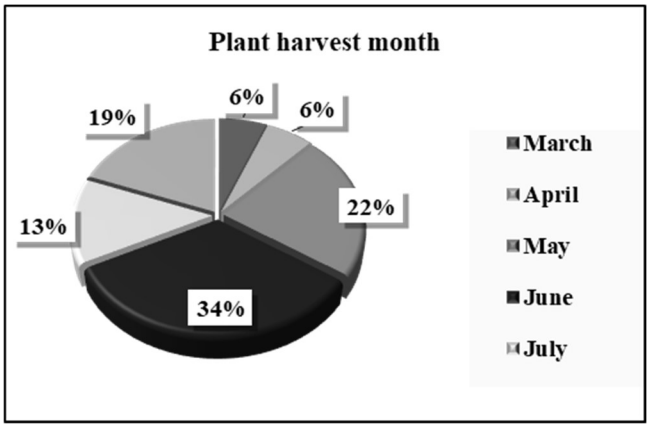

Figure 10. Preferred harvest season of *Pistacia lentiscus L.*

The months of May and June were determined as the preferred season for harvesting according to survey results **(Figure 10)**. According to **Figure 11**, all therapists use herbal tea for some therapeutic uses. Where 41.67% treated patients with *P. lentiscus L.* essential oils. Extracts were only guaranteed at 13.89% of them. Our results are consistent with what presented in study of Bammou M et *al*. [21], they mentioned that herbal tea was used most by traditional healers, and a majority used the extracts **(Figure11)**.

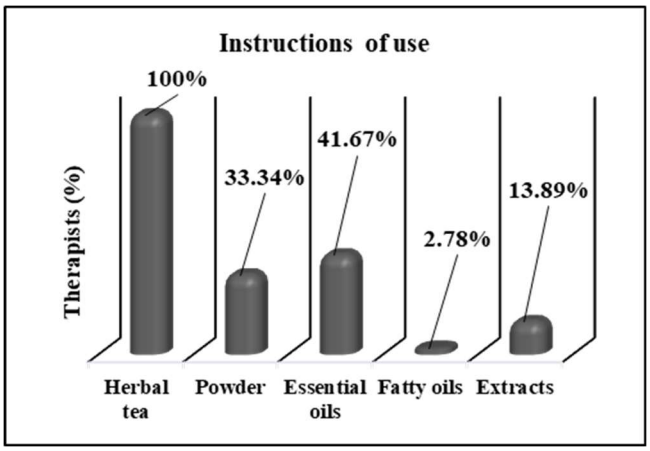

Figure 11. Modes of use of *Pistacia lentiscus L.*

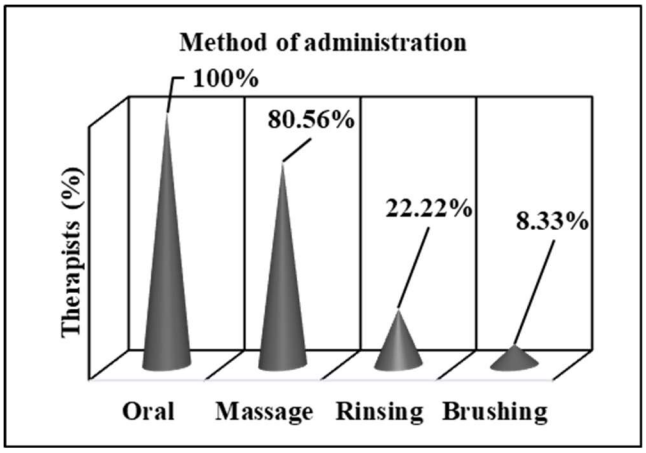

Figure 12. The rate of administration of *P. lentiscus L.*

Oral treatment (100%) and massage therapy (80.56%) are the two applied methods most frequently used by therapists **(Figure 12)** when it has been determined that 40% of the therapy was for external use.

The oral routes identified three different modes of use, with a total percentage equivalent to 60% **(Figure 13)**. Among the most important results of this ethnobotanical study on the *lentiscus* is its wide use in treating many diseases or their symptoms. Among the diseases that were treated with the plant through the digestive tract, we recorded 24%, both for gastric ulcers and abdominal pain [15, 41]; regarding certain chronic metabolic diseases such as

diabetes (7%) and arterial hypertension (8%) **(Figure 14)** [38, 43-46].

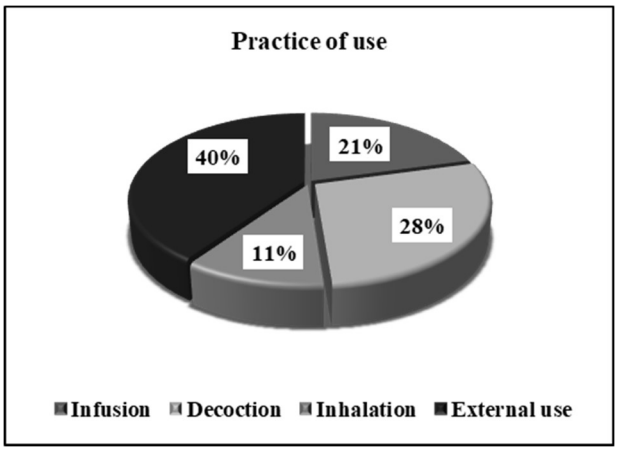

Figure 13. Different treatment practices with *Pistacia lentiscus L.*

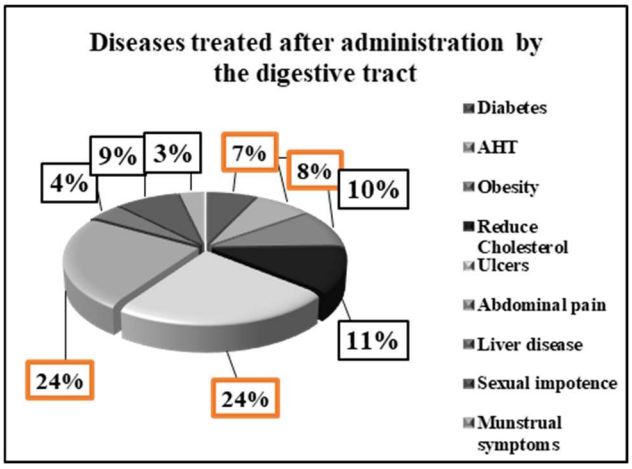

Figure 14. Diseases treated after administration of *Pistacia lentiscus L.* by the digestive route.

Pistacia lentiscus L. is not limited to this, but some therapists see that it as a solution to breathing problems and calming the nerves. It was via the respiratory tract, as summarized in the graphical representation **(Figure 15)**. Concerning this class of diseases, its treatment with *P. lentiscus L.* was mentioned in previous official scientific research [6].

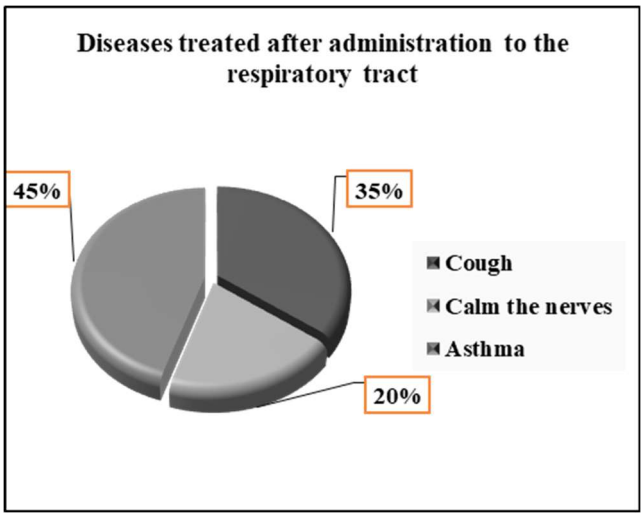

Figure 15. Diseases treated by *Pistacia lentiscus L.*

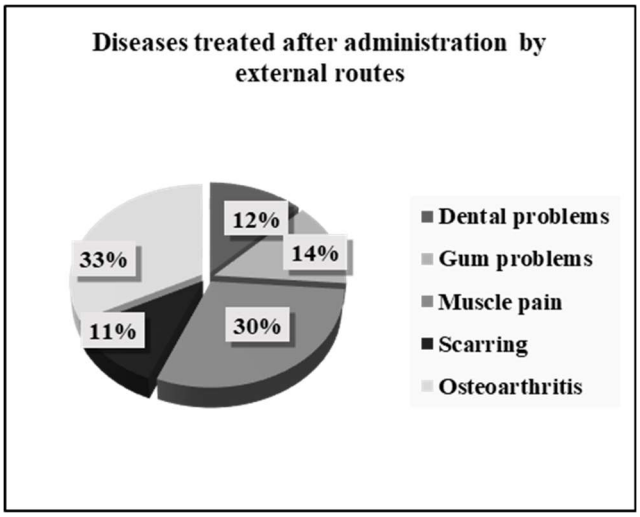

Figure 16. Symptoms and diseases treated after administration of *Pistacia lentiscus L.*

In addition to the above, this plant has excellent efficacy in external treatments such as osteoarthritis and muscle pain 63% of the total treatments of the plant by the external use, when the latter was mentioned in previous research [47]. Recent studies have confirmed that certain metabolites of *P. lentiscus L.* have beneficial effects on the rapid healing of skin lesions [48]. Also, this plant has a positive effect in eliminating gum pain, as reported by therapists who were surveyed **(Figure 16)**. The plant

studied also has aesthetic results, which vary according to cosmetic use **(Figure 17)** [49].

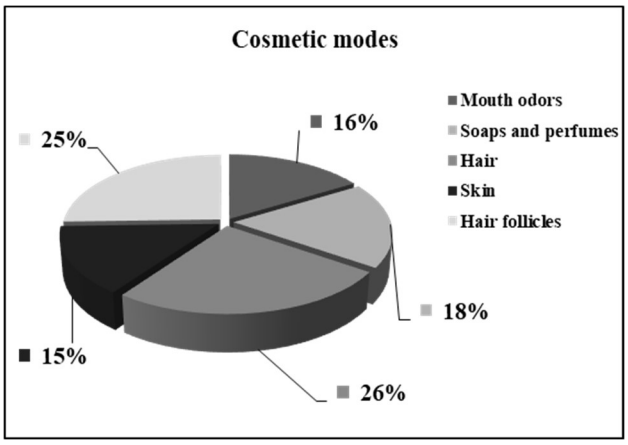

Figure 17. Cosmetic uses of the plant according to the therapists surveyed.

Characterization of the methanolic extraction

Table 1 summarized the methanol extraction yield and the physical characteristics of them. The yield of the methanolic extract of *P. lentiscus L.* was (37.1% and 32.4%) for Mersra and Mohammadia varieties respectively. It is corresponding to that obtained during a previous study (41.83%) [50] and (36.03%) [51]. This is due to many control parameters such as genetic characteristics, type of soil, geographical area and climate in particular and the method of extract preparation.

Qualitative phytochemical screening

The qualitative study of the biochemical compounds present in the structural tissues of the leaves of the *Pistacia lentiscus L.* plant harvested in the two previously mentioned regions allowed us to determine the presence of a considerable amount of secondary metabolites with biological activity (tannins, flavonoids, free anthraquinones, saponins, steroids, terpenoids, reducing compounds)**,** While, we recorded the absence of two types of organic chemical functions represented in Anthocyanins, sterols and triterpenes**,** with a weak positive result recorded for the last two compounds in the sample from the Mesra region **(Table 2).** The leaves of *P. lentiscus L.* are very rich in metabolites, especially with regard to polyphenols, flavonoids, tannins and terpenoids [52-54]. Qualitatively, the plant's exact composition of structural elements and chemical functional classes was recorded in both regions.

Table 1. The physical characteristic of the methanolic extract of *Pistacia lentiscus L.* harvested from two regions in Northwest Algeria.

Methanolic extract.	Mesra (Mostaganem)	Mohammadia (Mascara)
Yield (%)	37.1 ± 1.05	32.4 ± 0.75
Color	Dark green	Dark green
Aspect	Pasty	Pasty

Table 2. Chemical composition of the methanolic extract of leaves of *Pistacia lentiscus L.* harvested from two regions in North West of Algeria.

Chemical compounds	Varieties	
	Mesra	Mohammadia
Tannins	++	++
Flavonoids	++	++
Free anthraquinones	++	++
Anthocyanins	-	-
Saponins	+	+
Sterols and triterpenes	-/+	-
Steroids	+	+
Terpenoids	+	+
Reducing compounds	+	+
Alkaloids	+	+

++ **:** Moderately positive reaction
+ **:** Weakly positive reaction
-/+ **:** Very weak positive reaction
- **:** Negative reaction

Quantitative phytochemical study

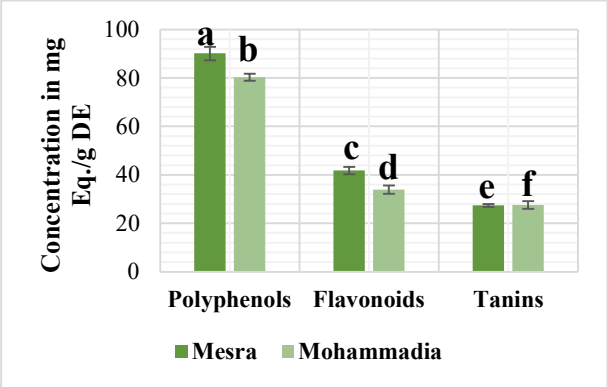

Figure 18. Comparison of total polyphenols, flavonoids and tannins amount in *Pistacia lentiscus L.* of Mesra and Mohammadia varieties

The quantitative study of the biologically active compounds present in the *Pistacia lentiscus L.* plant leaves was performed with samples harvested in two different regions. According to **Figures 19a, 19b** and **19c, t**he results showed an important content for polyphenols and flavonoids. A high content of polyphenols and flavonoids was shown in methanolic extracts of Mesra *Pistacia lentiscus L.* (Mostaganem) (90.12 ± 2.74 mg EqGAc/g DW and 41.86 ± 1.52 mg EqCer/g DW respectively), compared to those of Mohammadia (Mascara) (80.31 ± 1.42 mg EqGAc and 33.92 ± 1.71 mg Eq Cer/g DW) respectively with a significant difference ($P < 0.05$) **(Figure 18)**. Many studies confirm the presence of these secondary metabolites in *Pistacia lentiscus* [38, 52, 54-58]. Also, an extensive convergence with a non-significant effect was recorded in the content of condensed tannins for the two varieties of *P. lentiscus L.* of Mascara and Mostaganem (27.45 ± 056 mg EqCat/g DW and 27.61 ±1.53 mg EqCat/g DW) respectively **(Figure 18)**. The tannins are also essential compounds in *Pistacia lentiscus L.* [53, 59].

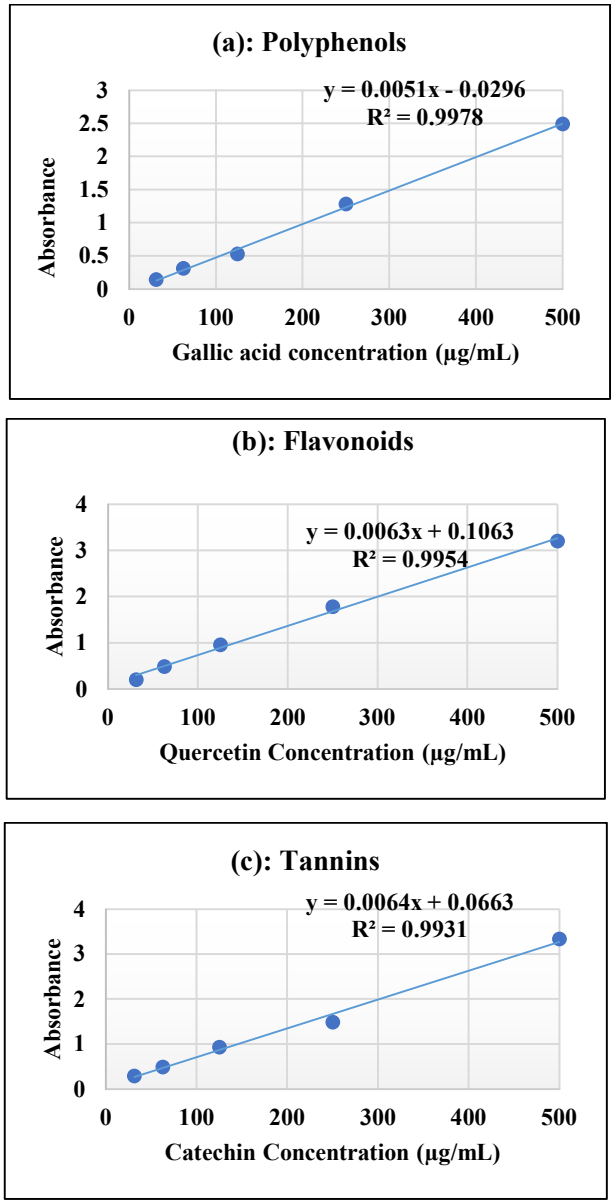

Figure 19. Calibration curve of absorbance determining the equivalent dose of polyphenols (a), flavonoids (b) and tannins (c).

Antioxidant activity and determination of IC_{50}

The results shown in **Figure 22** revealed the power of the methanolic extract of *P. lentiscus* of the two varieties from Mesra (***Fig. 22 a***) and Mohammadia (***Fig. 22 b***) to reduce the

free radical's DPPH. So, methanolic extract of *P. lentiscus L.* presented an important antioxidant potent (96.63% for Mesra and 95.49% for Mohammadia at concentration 1.25 mg/mL). Graphically, the IC_{50} value was determined as 0.06 mg/mL and 0.1 mg/mL for methanolic extracts of Mesra and Mohammadia varieties respectively.

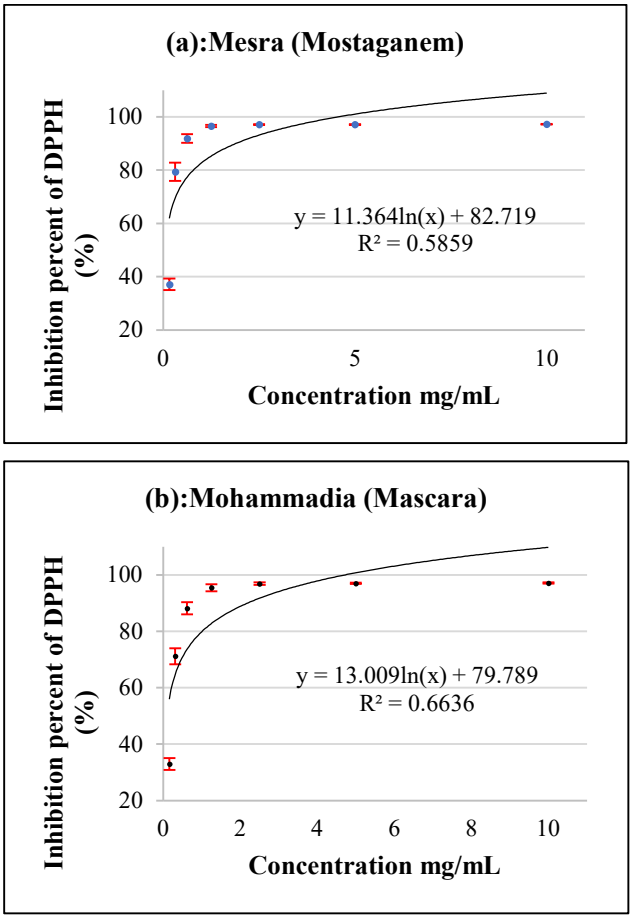

Figure 22. Inhibition percent of free radical DPPH of the methanolic extract of *Pistacia lentiscus L.* (a) Mesra (Mostaganem) (b) Mohammadia (Mascara).

The antioxidant capacity of phenolic compounds results from the high redox potential and the ability to eliminate electron or hydrogen atoms from free radicals, which causes a break in the reaction chains, which then generates oxidative stress [60]. The IC_{50} value was more effective for Mesra extracts than for Mohammadia extracts. These results were in accordance with previous studies as (0.08 mg/mL) in Morocco, 2022 [53], (0.12 mg/mL) in Blida, Algeria 2018 [51], and (0.05 mg/mL) in Setif, Algeria 2011 [61].

Although the antioxidant activity of the standard ascorbic acid was higher with lower concentrations **(Figure 23)**, relative values were recorded from the concentration of 1.25 mg/mL using the methanolic extract of *Pistacia lentiscus L.* from Mesra and Mohammadia cities **(Figure 24)**. These activities reflect an influence by certain factors such as the type of solvent and its polarity, the system used. It is therefore essential to make a global determination of the different antioxidants present in the plant to implement the various antioxidant mechanisms [62,63].

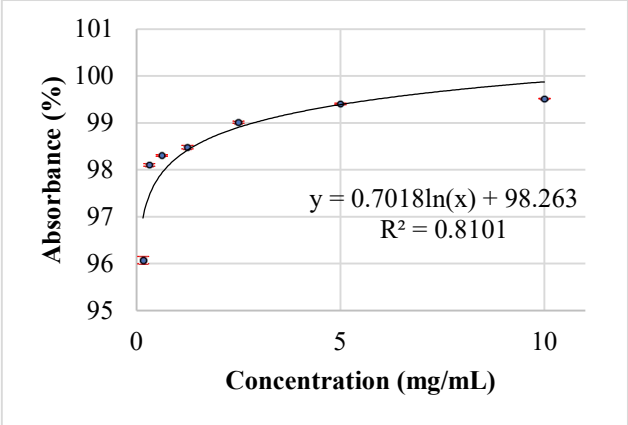

Figure 23. Antioxidant activity of ascorbic acid (Vit C)

Statistically, the results showed a significant difference in the antioxidant effect between the three substances ($P < 005$). So, this comparison confirmed that ascorbic acid was a potent antioxidant, often classified in group a. While the antioxidant activity of *Pistacia lentiscus L.* extracts was mainly in line with the

conventional reference (vitamin C) from 0.31 to 10 mg/mL. Also, a significant difference ($p < 0.05$) was recorded between the two methanolic extracts of the plant's regions. Where the most antioxidant power was recorded in Mesra extracts compared to Mohammadia, in the concentration range between 0.31 mg/mL and 1.25 mg/mL **(Figure 24).**

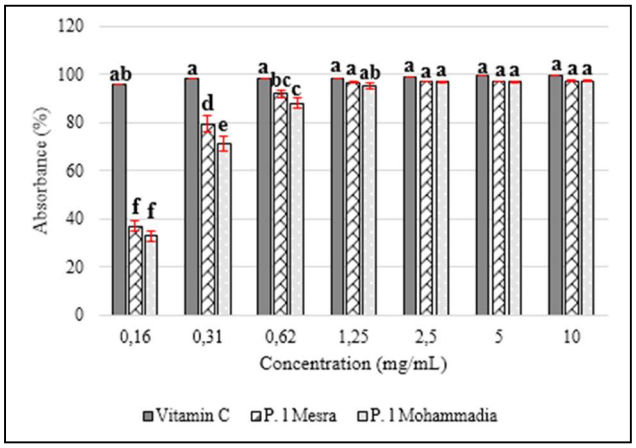

Figure 24. Comparison of the antioxidant activity of *P. lentiscus L.* with that of ascorbic acid.

P. l Mesra: Methanolic extracts of the leaves of *Pistacia lentiscus L.* harvested from the Mesra region.

P. l Mohammadia: Methanolic extracts of the leaves of *Pistacia lentiscus L.* harvested from the Mohammadia region.

Conclusion

Humans need to use natural substances characterized by practical biological activities. Our research targeted *Pistacia lentiscus L.* plant, a well-known medicinal plant in traditional medicine in Algeria, especially in the Northwest region. The ethnobotanical study has shown that a very high percentage of therapists have used this plant in their therapeutic performance. Many diseases or their symptoms were treated with *this medicinal and aromatic* plant. The phytochemical screening of the methanolic extract of *P. lentiscus L.* and the quantitative one, recorded for the two varieties from Mesra and Mohammadia, revealed their richness in secondary metabolites such as polyphenols, flavonoids and tannins. The methanolic extracts of the two varieties from Mesra (Mostaganem) and Mohammadia (Mascara) have reduced the free radicals of the DPPH molecule. This plant was a powerful antioxidant with an interesting IC_{50}. Therefore, the traditional use of this plant and its antioxidant effect are justified and experimentally verified. However, more studies are required to identify all the active compounds present in *P. lentiscus* L. and their precise mechanisms of action that open the field of complementary biological studies.

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