



## BIOLOGICAL ACTIVITIES OF TAOUNATE'S PISTACIA LENTISCUS ESSENTIAL OIL

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### ABSTRACT

The present work aims to evaluate the biological activities of the essential oil extracted from *Pistacia lentiscus*'s leaves of Taounate. The minimum inhibitory concentration of this oil against of seven bacterial strains Gram+ and Gram- is performed by the macrodilution in liquid medium. The antioxidant activity is determined by the diphenyl-picryl-hydrazyl (DPPH) test, and the anti-inflammatory activity is evaluated by using the plantar edema model induced in rabbit by the carrageenan. The results indicated that the foliar essential oil has an important antibacterial activity. *Mycobacterium smegmatis* is the most sensitive strain with a minimum inhibitory concentration (MIC) of 1/500. In addition, the results of the other tests showed that this oil has a low antioxidant and anti-inflammatory activity. The objective of this work has therefore been reached and the biological activities of the foliar essential oil of Taounate's *Pistacia lentiscus* are evaluated.

### Key words

*Pistacia lentiscus*; essential oil; antibacterial activity; antioxidant activity; anti-inflammatory activity; Taounate.

### INTRODUCTION

*Pistacia lentiscus*, is a 1 to 3 meters shrub, of the *Anacardiaceae*'s family (Leprieur, 1860). Particularly representative of the hottest areas in the Mediterranean climate. Locally called as 'Drou'; is known for its medicinal characteristics since the antiquity era. Its leaves have anti-inflammatory, antibacterial, antifungal and antipyretic activities. It also has astringent, hepatoprotective, expectorant and stimulating characteristics (Villar et al., 1987; Magiatis et al., 1999; Janakat and Al-Meir, 2002; Kordali et al., 2003; Paraschos et al., 2007). It also used in the treatment of eczema, oral infections, diarrhea, ephrolithiasis, jaundice, headaches, asthma and respiratory problems (Villar et al., 1987; Ali-Shtayeh et al., 1998; Ali-Shtayeh et al., 2000).

However; few researchers were interested to study the essential oils extracted from the leaves of *Pistacia lentiscus* from Taounate, a city situated north of Morocco. In fact, a prior study has highlighted the chemical composition of the essential oils extracted from leaves and branches of this plant (Hafsé et al., 2013). The results have shown that the essential oil extracted from leaves is rich of bioactive compounds (1.3% 1,8-cineol, 3.5%  $\alpha$ -terpineol, 1.2% linalol et 1.6%  $\alpha$ -pinene). It is, therefore, interesting to continue this work by determining the minimum inhibitory concentration, and evaluating the antioxidant and anti-inflammatory activity of this oil.

### MATERIAL AND METHODS

#### Plant material

*Pistacia lentiscus* was collected from Taounate (Altitude: 475m, north: 34° 35.203', West: 004°38.533'), the identification was conducted at the National Institute of Medicinal and Aromatic Plants of Taounate. The essential oil was obtained by hydrodistillation on a Clevenger-typ apparatus (Clevenger, 1928) (for each test, 100 g of fresh leaves were treated for three hours), then stored in optimal conditions (4°C in the dark in the presence of sodium sulphate anhydrous).

#### Bacterial strains

Several gram-positive and gram- negative bacteria were used: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus* sp., *Salmonella* sp., *Enterococcus faecalis*, *Mycobacterium smegmatis* MC<sup>2</sup>155 and *Mycobacterium aurum* A+ (Laboratory of microbial biotechnology of the Faculty of Sciences and Techniques. Fez, Morocco).

#### Antibacterial activity

Macrodilution in liquid medium was used to determine the minimum inhibitory concentration (MIC) of the foliar essential oil (EO), against the tested bacteria (Traoré et al., 2012). Because of the non-miscibility of the EO to water and thus to broth culture, the emulsification was carried out using 0.2% agar solution to foster germ/ compound contact. Dilutions of the EO were prepared at 1/10<sup>0</sup>, 1/25<sup>0</sup>, 1/50<sup>0</sup>, 1/100<sup>0</sup>, 1/200<sup>0</sup>, 1/300<sup>0</sup> and 1/500<sup>0</sup> in this agar solution. In test tubes each containing 9ml of sterile Luria-Bertani Broth, 1ml was added of each dilution to obtain the final concentration of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v/v). Each tube was seeded by 100  $\mu$ l of standardized inoculum of 10<sup>8</sup> CFU/ml and



incubated for one night at 37°C. The MIC of the EO was then determined. It corresponds to the concentration of the first tube in which there is a lack of growth of the tested germ that is visible by the naked eye. Controls corresponded to different dilutions not inoculated.

### Antioxidant activity

The estimation of the antiradical power of the essential oil was carried out by the 1, 1-diphenyl-picryl-hydrazil (DPPH) method according to Amzouar et al., (2013) method. Dilutions of the EO ranging from 20 to 0.03 mg/ml were prepared in methanol.

A mixture of 1.5 ml of the DPPH methanolic solution (0.04%) and of 1.5 ml of each concentration of the tested OE was prepared and was vigorously vortexed. Tubes were incubated at ambient temperature and in dark for 30 min. The negative control was composed of 1.5 ml of the methanolic solution of DPPH (0.04%) and 1.5 ml of methanol. The positive control corresponded to the methanolic solution of the BHT (20 to 0.03 mg/ml) and 1.5ml of the DPPH methanolic solution (0.04%). The absorbance (Abs) was measured at 517 nm. The antioxidant activity was estimated according to the following equivalent:

$$\% \text{ antioxidant activity} = (\text{Abs control} - \text{Sample Abs} / \text{Abs control}) \times 100$$

### Anti-inflammatory activity

The assessment of the anti-inflammatory activity was carried out by the inhibition of the plantar edema in the rabbit (*Oryctolagus cuniculus* Males and Females, 1900-2000 g), these rabbit were individually placed in a transparent Plexiglass cage (2000 cm<sup>2</sup>, 30 cm) in observation chamber. A technique inspired by those described by Winter et al., (1962) and Adeyemi et al., (2002).

The rabbits were fasted for 16 hours before the treatment and divided into four groups of three rabbits in each. Group 1 received NaCl 0.9% (10 mg/kg PC), groups 2 and 3 were respectively treated with 0.5% and 0.1% of the essential oil of *Pistacia lentiscus*. In order to compare the effect of the studied essential oil, the rabbits of group 4 were treated with the indomethacin at a dose of 300 mg/kg PC. The whole of these products were applied by a local massage for 30 min before the injection of the carrageenan 1% (0,7 ml). The evaluation of the edema was followed by recording of the leg volume for 0, 1, 2, 3, 4 and 5 hours after the injection of phlogogenic agent. For each treated group, average volumes obtained in these different readings ( $V_t$ ) were compared with those obtained before any treatment ( $V_0$ ), allowing to calculate the edema percentage (the inflammation percentage), from the following formula:

$$((V_t - V_0) / V_0) / 100$$

Whereas the percentage of inhibition of the edema was calculated from the formula:

$$[(V_t - V_0) \text{ control} - (V_t - V_0) \text{ treated}] / (V_t - V_0) \text{ control} * 100$$

### Statistical analysis

The results were expressed by the average and standard deviation. The comparison of the averages was carried out by the student's test and was considered significant with a value of  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Antibacterial activity

Table 1 presents the minimum inhibitory concentrations of the foliar EO of *P. lentiscus*. The results indicate that the foliar EO is active against the seven tested bacteria at a dose of 1/125, and that the antibacterial effect of this oil is limited to *Bacillus* sp., *Staphylococcus aureus*, *Mycobacterium smegmatis* and *Mycobacterium aurum* at a dose of 1/250 and is absent at 1/1000. This result allows us to conclude that the antibacterial activity of the studied EO is dose-dependent.

The results show that *Mycobacterium smegmatis* is the most sensitive strain vis-a-vis the foliar EO with an MIC of 1/500. 1/250 is the minimum inhibitory concentration of *Mycobacterium aurum*, *Bacillus* sp. and *Staphylococcus aureus*. The most resistant strains are *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Salmonella* sp. with an MIC of 1/125.

The significant sensitivity of mycobacteria against the tested essential oil (MIC of *Mycobacterium smegmatis* is 1/500) confirms the obtained results by Sqalli et al., (2007) and Hafsé et al., (2013).

In addition, *Pseudomonas aeruginosa* with an MIC of 1/125 shows resistant, this resistance is confirmed by the results of preliminary studies (Benhammou et al., 2008 ; Derwich et al., 2010). Furthermore, the evaluation of the antibacterial activity of the *P. lentiscus* foliar EO against the same strain by the disk diffusion method has shown a significant sensitivity of *Pseudomonas aeruginosa* (17mm) (Hafsé et al., 2013). Generally, the more important the inhibition zone is, the lower the MIC. However, strains with the largest inhibition zone are not always the most sensitive bacteria to antimicrobials. (Gonçalves et al., 2009).

The chromatographic analyses of the foliar essential oil of *P. lentiscus* have revealed the presence of monoterpenes, 1,8-cinéol,  $\alpha$ -terpineol, linalol and  $\alpha$ -pinene (Hafsé et al., 2013), these constituents are recognized for their antiseptics and antimicrobials effects (Damjanovic, 2012). The 1,8-cineole is highly effective against *Salmonella* et *Staphylococcus* (Prudent et al., 1993; Aligiannis et al., 2000). In addition, the linalol which is a terpene alcohol is also known by its inhibitory activity against *Enterococcus* sp. and *E. coli* (Mazzanti G et al., 1998).



**Table 1. MIC of the foliar essential oil of *Pistacia lentiscus*.**

Bacteria	1/10	1/100	1/125	1/250	1/500	1/1000	1/2000
Enterococcus faecalis	-	-	-	+	+	+	+
Salmonella sp.	-	-	-	+	+	+	+
Staphylococcus aureus	-	-	-	-	+	+	+
Bacillus sp.	-	-	-	-	+	+	+
Pseudomonas aeruginosa	-	-	-	+	+	+	+
Mycobacterium Aurum	-	-	-	-	+	+	+
Mycobacterium smegmatis	-	-	-	-	-	+	+

**+Growth, - Absence of growth**

### Antioxidant activity

The evaluation of the antiradical activity of the *P. lentiscus* ethanolic extracts is carried out by different techniques; these extracts are active regardless of the techniques used (Benhammou et al., 2008). In addition, Gardeli et al., (2008) have evaluated the antioxidant activity of *Pistacia lentiscus* methanolic extracts which proved to be important with IC<sub>50</sub> that vary between 5.09 to 11 mg/l.

The range of the tested foliar essential oil of *Pistacia lentiscus* is 20 to 0.03 mg/ml. The results have indicated that the concentration of 20 mg/ml presents the highest antioxidant effect 18,44% (Table 2) and that I'IC<sub>50</sub> is approximately 72.02 mg/ml; This essential oil is therefore having a low antioxidant activity unlike the ethanolic and methanolic extracts of the same plant. This can be explained by the absence of the molecules responsible for the antioxidant activity in the essential oil, and their extraction is done only by the organic solvents. Akrou et al., (2011) have shown that the essential oil of *Artemisia campestris* has a low antioxidant activity, whereas the aqueous and organic extracts of the same plant have an important activity.

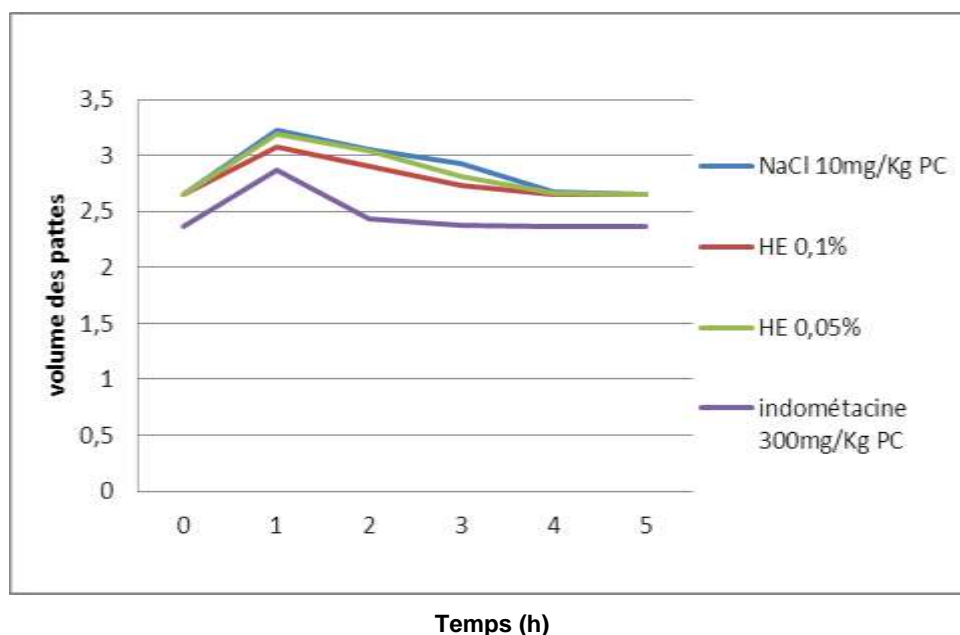
**Table 2. Optical densities and percentages of inhibition of the DPPH by the range of concentration of the foliar essential oil of *P. Lentiscus*.**

concentration mg/ml	DO	Scavenging power (%)
20	0,367	18,44
10	0,372	17,33
5	0,38	15,55
2	0,385	14,44
1	0,388	13,77
0,5	0,41	8,88

### Anti-inflammatory activity

To our knowledge, this study on the anti-inflammatory activity of the foliar EO of *P. lentiscus* is conducted for the first time. The variation in legs volumes in terms of time among the different treated groups is presented in Figure 1. The results of both concentrations of the essential oil tested are compared to those of indomethacin (Group 4), and to those of the control which received the physiological serum (Group 1).

One hour after the injection of carrageenan, the legs volumes in groups 2 (EO 0.05%) and 3 (EO 0.1%) are respectively  $3.18 \pm 1.2$  and  $3.07 \pm 0.2$  ml. By comparing these volumes with that of Group 4 ( $2.87 \pm 0.1$  ml), we can conclude that the indomethacin (300mg/kg PC) is more effective than the EO tested. However, the result shows that, compared to the control group 1 ( $3.22 \pm 1$ ml), the injection of the EO (0.1%) has decreased the inflammation; this decrease is translated by the legs volume of 3.07ml (Group 3).



**Figure 1. Evolution of the legs volumes in function of time among group 1 (NaCl 10 mg/kg PC), group 2 (EO 0.05%), group 3 (EO 0.1%) and Group 4 (indomethacin 300 mg/kg PC).**

## CONCLUSION

This work has revealed the important antibacterial activity of the foliar essential oil of *P. lentiscus* which depends on the studied dose and on the tested bacterial strain. However, this oil is having a low antioxidant and anti-inflammatory activity. *P. Lentiscus* is widely used by the population of Taounate, according to the results of the current work; its important antibacterial activity confirms its uses as an antiseptic for the treatment of digestive diseases and dermatological disorders. Nevertheless, the low anti-inflammatory and antioxidant effects of the foliar essential oil of this plant contradict its use for the treatment of osteoarticular disorders and its cosmetic use (against skin aging).

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## CONFLICT OF INTERESTS

There are no conflicts of interest

## REFERENCES

1. Adeyemi ,O.O., Okpo, S.O., Oguni, O.O. 2002. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea Americana* Mill (Lawraceae). *Fitoterapia*, 73: 375-80.
2. Ali-Shtayeh, M.S., Yaghmour, R.M.R., Faidi, Y.R., Salem, K.A.L., Al-Nuri, M.A. 1998. .Antimicrobial activity of 20 plants used in folkloric medicine in the palestini an area. *J. Ethnopharmacol.* 60: 265-271.
3. Ali-Shtayeh, M.S., Yaniv, Z., Mahajna, J. 2000. Ethnobotanical survey in the palestini an area: a classification of the healing potential of medicinal plants. *J Ethnopharmacol*, 73: 221-232.
4. Amezouar, F., Badri, W., Hsaine. M., Bourhim. N., Fougrach, H. 2013. Evaluation des activités antioxydante et anti-inflammatoire de *Erica arborea* L. du Maroc. *Pathologie Biologie.* 61 : 254–258.
5. Benhammou, N., Bekkara, F.A., Panovska, T.K. 2008. Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts. *Afr J Pharm Pharmacol.* 2: 22-28.
6. Clevenger, J.F. 1928. Apparatus for the determination of volatile oil. *J Am Pharm Assoc.*, 17: 346-341.
7. Damjanovic, B.M. 2012. Chemical composition and antibacterial activity of two *Juniperus* species essential oils. *Afri J Microbiol Res.*; 6 (38): 6704-6710.



8. Derwich, E., Manar, A., Benziane, Z., Boukir, A. 2010. GC/MS Analysis and In vitro Antibacterial Activity of the Essential Oil Isolated from Leaf of *Pistacia lentiscus* Growing in Morocco. *Sci J. 8* (10): 1267-1276.
9. Gonçalves, S., Quintas, C., Gaspar, M.N., Nogueira, J.M.F., Romano, A. 2009. Antimicrobial activity of *Drosophyllum lusitanicum*, an endemic Mediterranean insectivorous plant. *Nat Prod Res.*; 23:219-229.
10. Hafsé, M., Fikri Benbrahim, K., Saidi, A., Farah, A. 2013. Volatile Components and Antibacterial Profile of Essential Oils Extracted from Leaves and Twigs of *Pistacia lentiscus* L. *British Microbiology Research Journal. 3*(4): 602-611.
11. Janakat, S., Al-Merie, H. 2002. Evaluation of hepatoprotective effect of *Pistacia lentiscus*, *Phillyrea latifolia* and *Nicotiana glauca*. *J of Ethnopharmacolo. 83*: 135-138.
12. Kordali S, Cakir A, Zengin H, Duru ME. 2003. Antifungal activities of the leaves of three *Pistacia* species grown in turkey. *Fitoterapia. 74*:164-167.
13. Magiatis, P., Melliou, E., Skaltsounis, A.L., Chinou, I.B., Mitaku, S. 1999. Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. *chia*. *Planta Med. 65*: 749-751.
14. Paraschos, S., Magiatis, P., Mitaku, S., Petraki, K., Kaliaropoulos, A., Maragoudakis, P., Villar, A., Sanz, M.J., Payo, M. 1987. Hypotensive effect of *Pistacia lentiscus* L. *Int J Crude Drug Res. 25*: 1-3.
15. Sqalli, H., El Ouarti, A., Ennabili, A., Ibensouda, S., Farah, A., Haggoud, A., Houari, A., Iraqui, M. 2007. Evaluation de l'effet antimycobactérien de plantes du centre-nord du Maroc. *Bull Soc Pharm Bordeaux. 146*: 271-288.
16. Traoré, Y., Ouattara, K., Yéo, D., Doumbia, I., Coulibaly, A. 2012. Activités antifongique et antibactérienne des feuilles d'*Annona senegalensis* Pers. *J Appl Biosci. 58*: 4234-4242.
17. Villar, A., Sanz, M.J., Payo, M. 1987. Hypotensive effect of *Pistacia lentiscus* L. *Int J Crude Drug Res. 25*: 1-3.
18. Winter, C.A., Risley, E.A., Nuss, G.W. 1962. Carrageenin-induced edema in the hind paw of rat as an assay for anti-inflammatory drugs. *Proc Soc Biol Med. 11*: 544-547.



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