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ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF *PISTACIA ATLANTICA* AGAINST *ASCOCHYTA RABIEI* AND ITS CORRELATION WITH ANTIOXIDANT ACTIVITY

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Abstract

The essential oil of the leaves of *Pistacia atlantica* has biological activities known since antiquity especially in Africa. This work targets the study of the correlation between the antifungal activity against *Ascochyta rabiei* and the antioxidant activity of this essential oil. After obtaining the oil by the hydrodistillation process, a chemical characterization was carried out by GC-MS, the latter makes it possible to identify thirty-eight compounds (7.66%), among them four in the majority which are: terpinen-4-ol (17.04%), α-pinene (16.03%), α-thujene (6.24%) and spathulenol (5.05%). The antifungal activity was achieved by the method of growth of fungie colonies on agar medium, the antioxidant activity was achieved in vitro by means of the DPPH free radical scavenging test and the correlation was made by a statistical study of trend curves according to three models: linear, exponential and third-degree polynomial. This last model confirmed the liaison between the two activities from the value of coefficient of determination R^2 = 72.72%.

Keywords: *Pistacia atlantica, Antifungal activity, Antioxidant activity, correlation, trend curves.*

Introduction

Ascochyta blight, a microbial disease caused by the fungus *Ascochyta rabiei* (Pass.) Labrousse, is the major problem for chickpea (*Cicer arietinum* L.) production in all countries of the world **[1]**. Current farmers have only partial resistance to the pathogen through the use of chemical fungicides, and this level of resistance can easily degrade because the pathogen is highly variable due to the potential for sexual recombination **[2-4]**. The development of fungicidal products can control the disease and consequently the successful production of chickpeas **[5-6]**.

Several fungicidal products have been reported in various scientific works which have a high efficiency effective in treating ascochyta blight, but their application limits remain irreversible because of low production yield **[7-8]**. Several synthetic products are considered effective in preventing the secondary spread of ascochyta blight, among them: penconazole, antracol, captan, chlorothalonil, propiconazole, maneb, thiabendazole, as well as sulfur-based fungicides **[9-12]**. On the other hand, several natural products based on plant extracts or essential oils have been tested and found to be effective against many species of Ascochyta **[13]**. Plants have secondary metabolites with antifungal activity against this fungus, are: *Salvia officinalis*, *Salvia tomentosa*, *Myrtus communis*, *Melaleuca alternifolia*, *Mentha spicata*, *Thymus vulgaris*, ... **[14-16]**. Likewise, other biological control agents against ascochyta blight have been reported and which have been shown to be particularly effective in vitro: *Chaetomium globosum*, *Trichoderma viride*, *Acremonium implicatum*, ... **[17-19]**.

This study is the continuation of several works of our team aimed at evaluating the interest in the protection of leguminous crops, essential oils whose antifungal and antibacterial properties are already known and used in human and veterinary medicine, with the perspective of offering to farmers partial or total alternatives to the products currently in use **[20-25]**. Even if it is possible to think that the reduction in the use of fungicides will be done by mobilizing instead agronomic levers, new bio-sourced fungicidal solutions are nevertheless to be sought within the framework of organic and conventional agriculture. The present work consists of testing in vitro the power of the essential oil of *Pistacia atlantica* against the fungal disease of Ascochyta blight and on the other hand, to know the correlation between this activity and the antioxidant activity via DPPH radical scavenger assay.

Materials and methods

Plant material

The leaves of *Pistacia atlantica* were collected from the wild around Khenifra, in the middel Atlas of Morocco in July 2020. A voucher specimen (PA347/2020) was deposited in the herbarium of the EST-Khenifra (University of Sultan Moulay Slimane). The plant material was dried in the laboratory under normal air and at room temperature conditions.

Extraction of essential oil

The essential analyzed oil in this study was extracted by hydrodistillation of *Pistacia atlantica* leaves (1Kg) for 3 h using a Clevenger-type apparatus. The essential oil was performed in triplicate. The oil was extracted with diethyl ether (3×100 ml) and dried with anhydrous Na2SO4. For yield determination, the solvent was evaporated using a rotavapory vacuum evaporator, the yield was calculated according to dry weight of the plant material. The resulting oil was transferred to opaque glass and stored at 4°C during the experimental period **[26]**.

GC–MS analysis

For the analysis of *Pistacia atlantica* essential oil an Agilent 6890N-5973 N GC–MS (Palo Alto, CA, USA) system equipped with a HP-5MS capillary column (30 m x 0.25 mm i.d., 0.1 µm f.t.). The analytical conditions employed were those previously cited by Ainane et al. (2021). The assignment of the peaks relied on the use of temperature-programmed retention indices (RIs) and mass spectra (MS) fragmentations and their comparison with those stored in some libraries (ADAMS, FFNSC2, NIST17 and WILEY275). Moreover, the comparison with available analytical standards was also used. Peak area relative percentages were obtained from the chromatograms setting the same response factor for all chemical classes occurring in the essential oil **[27]**.

Antifungal activity

The pure culture of the test fungal species *Ascochyta rabiei* was obtained from the Regional Center for Agronomic Research in Settat (Morocco). The culture was maintained on agar medium with malt extract (MEA). The method used for antifungal activity is described by Jabeen & Javaid (2010) **[28]**. Malt extract agar (MEA) medium was prepared and cooled to 50°C. Appropriate quantities of stock solution of essential oil and distilled water were added to MEA medium to get 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5% (v/v) final concentrations in the medium. Control received the same quantity of distilled water. The all products were thoroughly mixed with the medium. Twenty ml of each medium was poured in each 9 cm diameter sterilized Petri plate. Mycelial discs of 5 mm diameter were taken with a presterilized cork borer from 5-7 days old culture of *Ascochyta rabiei* and were placed in the center of each Petri plate after solidification of the MEA medium. Each treatment was replicated thrice. Plates were incubated in an incubator at 25±2°C for 7 days. Fungal growth was measured by averaging the 3 times diameters taken at right angles for each colony.

Antioxidant activity

Antioxidant activity was done by the DPPH Radical Scavenging Assay. The activity was determined following the method of Attahar et al. (2021) **[29]**. The stock solution of the essential oil was prepared in methanol at the concentration of 20 % (v/v). fifty microliters of each extracts were added to 150 µL of DPPH radical solution in 96-well plates, in order to obtain final concentrations of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 %. The samples were shaken. Due to the colored extracts that can absorb at λ_{max}=520 nm, it was necessary to prepare a control (i.e. sample blank) which consisted of 50 µL of each sample solution added to 150 µl of methanol. Then the absorbance of samples was measured at 60 min at 520 nm, with methanol as the blank, using spectrophotometer (Multiscan, Perkin Elmer). Ascorbic acid (Vit C) was used as standard. The DPPH radical activity was calculated using the following equation:

$$Scavenging\ effect\ (\%) = \frac{1 - (A_{sample} - A_{sample\,blank})}{A_{control}} \times 100$$

Results and discussion

The extraction of essential oil from the leaves of *Pistacia atlantica* was obtained as a yellow-green oil with an aromatic-spicy strong odor with a yield of 0.08% (v/w). The chemical composition of this oil was analyzed by GC-MS, it results in the identification of Thirty-eight compounds with a total percentage of 70.66%. The majority compounds identified are: terpinen-4-ol (17.04%), α-pinene (16.03%), α-thujene (6.24%) and spathulenol (5.05%) (Table 1).

Barrero et al. (2005) **[30]** worked on essential oils from different parts of *Pistacia atlantica* (Resins, fruits and leaves) from the High Atlas of Morocco, hence the chemical composition of the oil of the leaves showed a very high percentage of sesquiterpene compounds such that: terpinen-4-ol (21.7%) and elemol (20.0%).

Tzakou et al. (2007) **[31]** did a study to identify essential oils from leaves of *Pistacia atlantica* and separate the chemotypes of male and female trees. In male plants, essential oil from male plants contains p-mentha-1(7)-8-diene as a major compound along with other predominant compounds such as: terpinene-4-ol, sabinene and α-pinene. The essential oil of the leaves of female plants consist mainly of monoterpene compounds such as: myrcene, sabinene and terpinene-4-ol being considered the major compound.

Gourine et al. (2010) **[32]** selected 34 samples of *Pistacia atlantica* leaves collected in different regions of Algeria. He showed that the essential oils of the leaves are rich in monoterpenes and oxygenated sesquiterpenes, the main major compounds are: α-pinene, α-thujene, camphene, β-pinene, p- cymene, terpinene-4-ol and spathulenol.

The comparison of our work with these works cited above, confirms the existence of terpinene-4-ol as a predominant compound which characterizes the essential oil of the leaves of *Pistacia atlantica.*

The antifungal activity of the essential oil of the leaves of *Pistacia atlantica* is shown in Figure 1. The activity was expressed as the fungal colony diameter as a function of the concentration of the essential oil. Usually the antifungal activity (lowest diameter) will be high relative to the growth of the

oil concentration, it is noted that the high activity was mentioned at the concentration of 2% of the oil with a diameter of 5.1 mm. Several works have been mentioned the interest of extracts of *Pistacia atlantica* among them its essential oil against yeasts and fungi in particular: *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae* **[33]**.

The antioxidant activity of the essential oil of the leaves of *Pistacia atlantica* made by the method of measuring the percentage of the activity of the DPPH radical scavenging assay is displayed in figure 2. An interesting activity was displayed in during the growth of essential oil concentration. Several researchers have documented the antioxidant activity of different extracts from different parts of the *Pistacia atlantica* plant. The main phenolic compounds of fruits and leaves that have antioxidant properties are derivatives of benzoic acid, derivative of hydroxycinnamic acid and flavonoids, their activities appeared with low concentrations **[34-35]**.

The correlation between the two antifungal and antioxidant activities of the essential oil of *Pistacia atlantica* was made by the statistical study of the trend curves of two variables such as: Y is the fungal colony diameter (mm) and X is the DPPH radical scavenging activity (%).

The trend curves are modeled by three methods: linear modeling, exponential modeling and polynomial at degree 3 modeling (Figure 2). Model reliability is examined by determining coefficient of determination R^2. All the data are mentioned in Table 2. After these results, according to the comparison of the coefficients of determination, the degree 3 polynomial model gives a good correlation between the two parameters Y and X with an R^2 = 72.72%, that is to say a relationship confirmed by this model between antifungal activity against *Ascochyta rabiei* and antioxidant activity. The other two linear and exponential models have coefficients of determination of 42.10% and 41.52% respectively, which explains why these two models do not present a good interpretation of the correlation.

Conclusion

The essential oil of the leaves of *Pistacia atlantica* from the Khenifra region (Morocco) is a substance with important antifungal activity against *Ascochyta rabiei* and important antioxidant activity. These activities are due to its chemical composition which contains the major chemotypes, particularly terpinen-4-ol (17.04%), α-pinene (16.03%), α-thujene (6.24%) and spathulenol (5.05%). The correlation between the two activities by statistical modeling of the parameters Y: fungal colony diameter and X: DPPH radical scavenging activity proves the existence of a link between them according to the 3rd degree polynomial model represented by the equation $Y = 0.0044X^2 - 0.2141X + 7.4794$ at a coefficient of determination of R^2=72.72%.

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Table 1. Chemical composition of essential oil of leaves of *Pistacia atlantica* from Khenifra (Morocco).

Peak	Component	(%)
1	Tricyclene	1.77
2	α-Pinene	**16.03**
3	α-thujene	**6.24**
4	Camphene	3.88
5	β-Pinene	1.00
6	Limonene	1.06
7	β-Phellandrene	0.02
8	*trans*-2-hexenal	0.05
9	γ-Terpinene	0.01
10	*p*-Cymene	0.04
11	α-Terpinolene	0.02
12	6-Methyl-5-hepten-2-one	0.02
13	(*E*)-3-hexen-1,ol	0.01
14	β-Thujone	0.08
15	Bornylene	2.02
16	Linalool	0.07
17	Bornyl acetate	0.02
18	Terpinen-4,ol	**17.04**
19	Aromadendrene	0.05
20	Alloaromadendrene	0.02
21	(*E*)-pinocarveol	1.64
22	Ledene	0.04
23	Linalyl propionate	1.15
24	α-Terpineol	2.52
25	α-Terpenyl acetate	1.51
26	Germacrene B	0.08
27	Geranyl acetone	1.35
28	Palustrol	0.02
29	Epiglobulol	1.47
30	Ledol	0.01
31	Globulol	0.01
32	Viridiflorol	0.01
33	Spathulenol	**5.05**
34	γ-Eudesmol	0.02
35	Isospathulenol	2.82
36	Phytol	1.02
37	Myristic acid	0.03
38	Palmitic acid	2.46
Total identified		**70.66**

Table 2. Model parameters of trendlines.

Model	Equation	R^2 (%)
Linear	Y = -0.0534X + 6.5026	42.10
Exponential	$Y = 6.4311e^{-0.009X}$	41.52
Polynomial (degree 3)	$Y = 0.0044X^2 - 0.2141X + 7.4794$	72.72

Figure 1. Effect of essential oil of *Pistacia atlantica* on in vitro growth of *Ascochyta rabiei*.

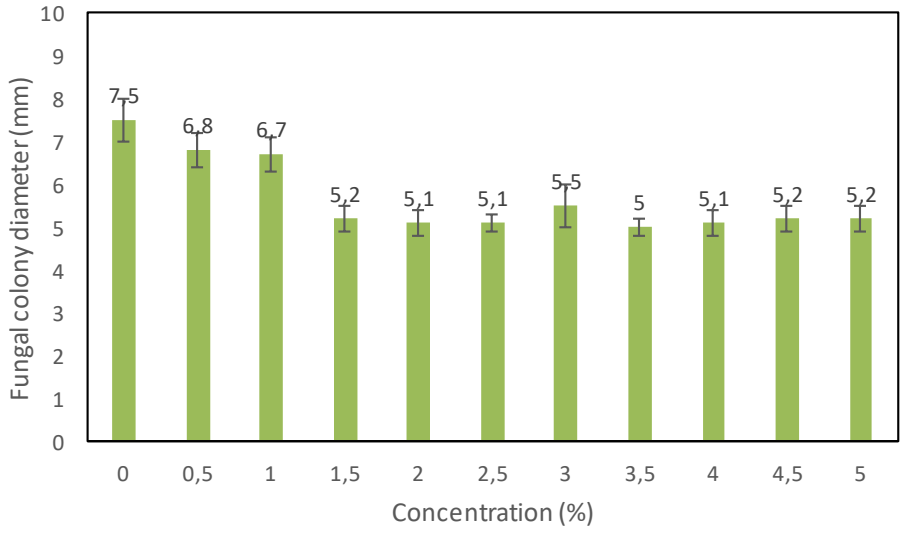

Figure 2. Antioxidant activity of different concentrations of essential oil performed by DPPH radical scavenger assay.

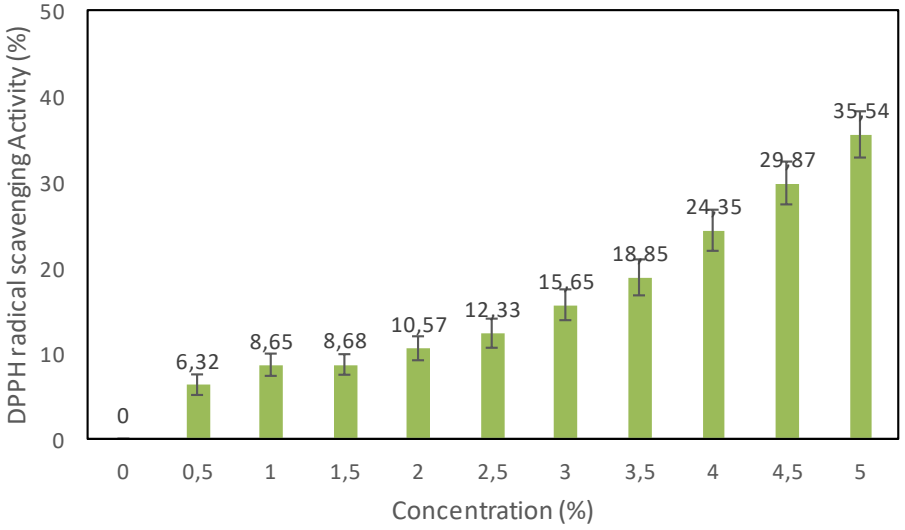

Figure 3. Modeling of the correlation between the antifungal activity and the antioxidant activity of the essential oil of *Pistacia atlantica*.