Antioxidant activity and content of total phenols and flavonoids of aqueous extracts from leaves of *Pistacia lentiscus* L.

Atividade antioxidante e conteúdo de fenóis e flavonóides totais de extratos aquosos de folhas de *Pistacia lentiscus* L.

Actividad antioxidante y contenido de fenoles y flavonoides totales de extractos acuosos de hojas de *Pistacia lentiscus* L.

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Benchiha Walid
pHD in Ecology at Relizane University
Relizane. Algeria
walid.benchiha@univ-relizane.dz

Aouadj Sid Ahmed
pHD in Ecology at Relizane University
Laboratory of Ecology and Management of Natural Ecosystems
Tlemcen. Algeria
sidahmed.aouadj@univ-relizane.dz

Megharbi Ahmed
pHD in Ecology at Relizane University
Relizane. Algeria
ahmed.megharbi@univ-relizane.dz

Tamert Asma
pHD in Ecology at Relizane University
Relizane. Algeria
asma.tamert@univ-relizane.dz

Lazreg Khaoula
student in Ecology at Relizane University
Relizane. Algeria
khaloula.lazeurg@univ-relizane.dz

Lazreug Nour El Houda
student in Ecology at Relizane University
Relizane. Algeria
nourelhouda.lazreug@univ-relizane.dz

Belkhadem Atika
student in Ecology at Relizane University
Relizane. Algeria
atika.belkhadem@univ-relizane.dz

ABSTRACT
The Mounts of Tessala (western of Algeria) is a remarkable site of plants diversity, characterized by the presence of numerous aromatic and medicinal species used by the local population as therapeutic agents, the mastic tree (*Pistacia lentiscus* L.) is among the plants inhabiting this mountain; it is a perennial shrub belonging to the family Anacardiaceae.
The dosage of some polyphenols (total phenols, flavonoids) varied respectively between 21.20 - 98.23 mg gallic acid equivalents/g and 39.767 - 91.288 mg catechin equivalents/g. ANOVA revealed a significant variation in the mean concentrations according to the used extract.
Evaluation of the antioxidant power of aqueous extracts has revealed a remarkable biological effect. The high inhibition percentages (100%) were found in the decocted extract at 10 mg/mL, followed by the macerated extract at 10 mg/mL, and finally the infused extract with 82.99% at 20 mg/mL. The DPPH free radical scavenging test revealed that the antioxidant activity of aqueous extracts varied significantly according to extract type and its concentration.
The results obtained not only confirm the richness of active principles in the studied plant but also its therapeutic effectiveness.

Keywords: *Pistacia lentiscus,* Total phenols, Flavonoids, antioxidant activity, Algeria.

RESUMO
Os Montes de Tessala (oeste da Argélia) são um local notável pela diversidade vegetal, caracterizado pela presença de inúmeras espécies aromáticas e medicinais utilizadas pela população local como agentes terapêuticos, a aroeira (*Pistacia lentiscus* L.) está entre as plantas que habitam esta montanha ; é um arbusto perene pertencente à família Anacardiaceae.
A dosagem de alguns polifenóis (fenóis totais, flavonóides) variou respectivamente entre 21,20 - 98,23 mg equivalentes de ácido gálico/g e 39,767 - 91,288 mg equivalentes de catequina/g. A ANOVA revelou variação significativa das concentrações médias de acordo com o extrato utilizado.
A avaliação do poder antioxidante dos extratos aquosos revelou um efeito biológico notável. As elevadas percentagens de inibição (100%) foram encontradas no extrato decotado a 10 mg/mL, seguido do extrato macerado a 10 mg/mL e por fim o extrato infundido com 82,99% a 20 mg/mL. O teste de eliminação de radicais livres DPPH revelou que a atividade antioxidante dos extratos aquosos variou significativamente de acordo com o tipo de extrato e a sua concentração.
Os resultados obtidos confirmam não só a riqueza de princípios ativos da planta estudada, mas também a sua eficácia terapêutica.

Palavras-chave: *Pistacia lentiscus,* Fenóis totais, Flavonóides, actividade antioxidante, Argélia.

RESUMEN
Los Montes de Tessala (oeste de Argelia) son un sitio notable de diversidad vegetal, caracterizado por la presencia de numerosas especies aromáticas y medicinales utilizadas por la población local como agentes terapéuticos, el lentisco (*Pistacia lentiscus* L.) se encuentra entre las plantas que habitan esta montaña; es un arbusto perenne perteneciente a la familia Anacardiaceae.
La dosis de algunos polifenoles (fenoles totales, flavonoides) varió respectivamente entre 21,20 - 98,23 mg equivalentes de ácido gálico/g y 39,767 - 91,288 mg equivalentes de catequina/g. ANOVA reveló una variación significativa en las concentraciones medias según el extracto utilizado.
La evaluación del poder antioxidante de los extractos acuosos ha revelado un efecto biológico notable. Los porcentajes de inhibición altos (100%) se encontraron en el extracto decocido a 10 mg/mL, seguido del extracto macerado a 10 mg/mL y finalmente el extracto infundido con 82,99% a 20 mg/mL. La prueba de eliminación de radicales libres DPPH reveló que la actividad antioxidante de los extractos acuosos variaba significativamente según el tipo de extracto y su concentración.
Los resultados obtenidos no sólo confirman la riqueza de principios activos de la planta estudiada sino también su eficacia terapéutica.

Palabras clave: *Pistacia lentiscus,* Fenoles totales, Flavonoides, actividad antioxidante, Argelia.

1 INTRODUCTION

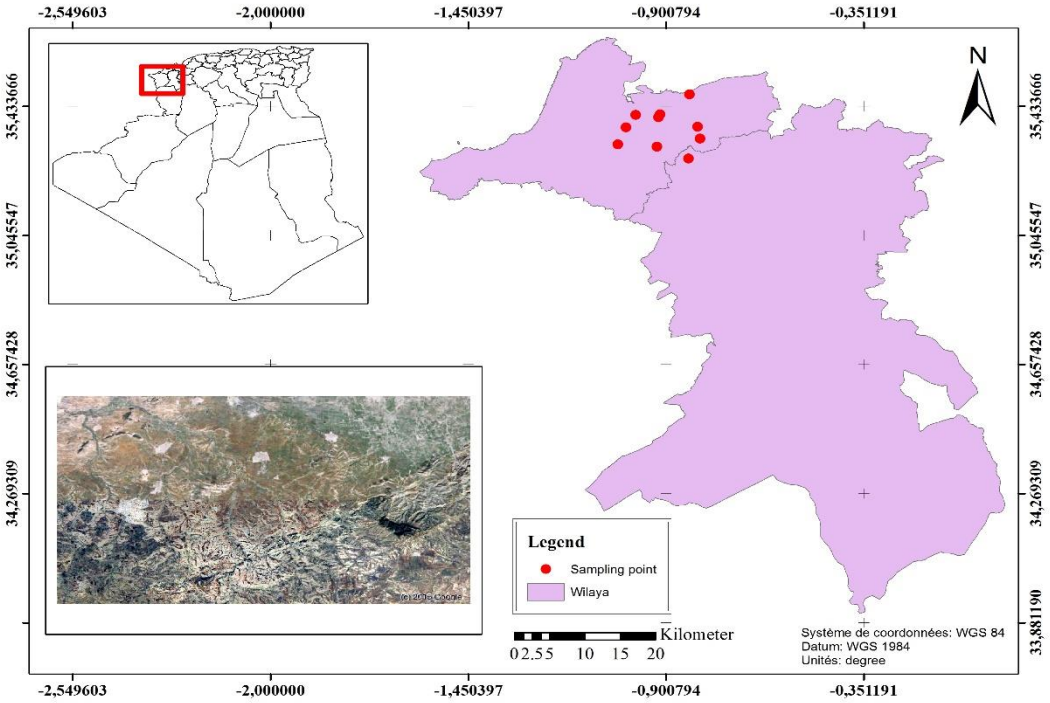
Medicinal plants have been an inexhaustible source of medicines for traditional practitioners to cure certain often fatal pathologies, without knowing what their beneficial actions were due to, administered either by friction, inhalations, poultices, massages or even orally (Lee, 2004 ; Aouadj et al., 2020a-f). They are considered an essential source of raw material for the discovery of new molecules necessary for the development of future drugs (Maurice, 1997). Currently, ethnopharmacology is working to identify plants known to be active throughout the world and for which it is up to modern research to specify the properties and validate the uses because it is estimated that around 80% of the planet's inhabitants use traditional herbal medicines as primary health care (Fleurentin & Pelt, 1990). The study of plant chemistry remains extremely relevant despite its age. This is mainly due to the fact that the plant kingdom represents an important source of bioactive molecules. Among the 250,000 to 300,000 inventoried species of plants found on earth, only 5% to 15% have been the subject of research for bioactive molecules, representing an immense reservoir of new potential medicinal compounds (Balandrin, 1993 ; Culter & Culter, 2000 ; Larson et al., 2007 ; Aouadj et al., 2020b-f)) and 6% for their biological activities (Verpoorte, 2002). Oxidative stress plays a major role in the aetiology of many diseases, such as atherosclerosis, arthritis, cardiovascular disorders, Alzheimer's disease and cancer (Halliwell, 1999). Free radicals are produced in the human body through normal metabolism or induced by physical and/or chemical factors in the environment. Harmful effects can be combatted by taking many natural antioxidants (Alimpic et al., 2014). Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxy-toluene (BHT), are very effective, but they can induce many side effects, such as tumours, at high doses and after long-term treatment (Kahl & Kappus, 1993). That's why there is increasing interest in antioxidants of natural origin which can be used in food, cosmetic and pharmaceutical products to replace synthetic antioxidants. For our part, our choice fell on the species Pistacia lentiscus L. which is a characteristic species of our region (western Algeria), it is a perennial shrub which grows almost throughout the region (Quezel & Santa, 1963). This plant is traditionally used in so many countries around the world and in Algeria.

2 METHODOLOGY

2.1 PLANT MATERIAL

The plant material was harvested in January 2024 at Mount of Tessala (Western Algeria: longitude W 0° 46'879", latitude N 35° 16' 370", altitude 967 m, south-west exposure) (Figure 1), the leaves were cleaned then dried in the shade and at ambient temperature for a period of 21 days then crushed using an electric grinder and preserved until their use figure 1.

Figure 1: The geographical localization of the study site (authors, 2024).



2.2 PREPARING THE EXTRACTS

In order to be following the traditional galenic form of use of the drug as recommended by traditional therapists, we carried out a decoction and maceration and an aqueous infusion to obtain the study extracts.

2.3 THE AQUEOUS DECOCTION

Twenty grams (20 g) of dry matter (leaf powder) were decocted in a flask containing 200 mL of distilled water; the decoction kept under continuous reflux for two hours. At the end of this operation, the decoction obtained after cooling was filtered through a funnel containing hydrophilic cotton.

2.4 THE AQUEOUS MACERATION

It was obtained under the same conditions as the decoction, the only difference being that here maceration (cold extraction) was carried out without any heating operation of the test portion (20 g).

2.5 AQUEOUS INFUSION

It was obtained by pouring boiling water over a precise quantity of plant material (20g). An infusion is prepared by letting the mixture sit for 10 to 15 minutes.

2.6 EVAPORATION OF THE SOLVENT

The three solutions obtained were evaporated using a drying oven at a temperature of 35°C for 24 hours, this process makes it possible to eliminate the solvent at the end of which the extracts recovered were weighed for to determine the yield.

2.7 DOSAGE OF TOTAL PHENOLS BY SPECTROPHOTOMETRY

A test portion of 50 µL of aqueous extract is diluted in 2.5 mL of distilled water, we add 250 ml of Folin-Ciocalteu reagent, the mixture is submitted to mixes by using vortex then we leave it for 5 minutes in ambient temperature. After stirring, 500 µL of 20% sodium carbonate are added. After stirring, 500 µL of 20% sodium carbonate are added. The tubes are then agitated well and then incubated at a temperature of 40°C for 30 minutes. The mixture is kept at ambient temperature and in the dark for 60 minutes.

The optical density is read with a UV-VIS spectrophotometer at a wavelength of 765 nm. A calibration curve produced by a standard (gallic acid) at different concentrations (0-100-200-300-400-500 mg/l) and carried out under the same operating conditions as those of the samples will be used for the quantification of total phenols (El Hadrami et al., 1997).

2.8 DOSAGE OF FLAVONOIDS

The quantification of the flavonoid content of the leaves of the plant is estimated by the aluminum trichloride (AlCl3) method.500 µL of aqueous extract are mixed with 1500 µL of distilled water and 150 µL of 5% sodium nitrate, we leave the mixture to rest for 5 min at ambient temperature and in the dark. This mixture is then added to 150 µL of 10% aluminum trichloride; after resting for 11 min in the dark, 500 µL of 1M sodium hydroxide is added. The mixture is subjected to vortex stirring and the optical density is read at a wavelength of 510 nm. A calibration curve produced by a standard (catechin) at different concentrations (0-10-20-30-40-50 mg/l) and carried out under the same operating conditions as those of the samples will be used for the quantification of total phenols (El Hadrami et al., 1997).

2.9 EVALUATION OF THE ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS

2.9.1 Measurement of anti-radical power by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test

The DPPH (2.2-diphenyl-1-picrylhydrazyl) method introduced by BLOIS (1958) is based on the reduction of an alcoholic solution of the stable radical species DPPH in the presence of a hydrogen-donating antioxidant, which results in the formation of a non-radical form, DPPH. The reduction induces a change from its purple color to yellow color (Maataoui et al., 2006). 750 µL of

extract are added to 1.5 mL of the freshly prepared DPPH solution in methanol. After standing for 30 minutes in the dark, the absorbance was measured at 515 nm against methanol as a blank. A control consisting of 750 µL of methanol and 1.5 mL of the DPPH solution is carried out with each series of extracts. The anti-radical activity of the extracts was expressed as a percentage of inhibition of the DPPH radical following the equation:

$$\% \text{ Inhibition} = ((\text{Control Abs} - \text{Sample Abs}) / \text{Control Abs}) \times 100$$

The results are compared to a reference antioxidant, in this case, ascorbic acid (vitamin C) (Molyneux, 2004). To overcome the influence of concentration, the inhibitory concentration IC50 of the antioxidant is determined. The latter corresponds to a 50% reduction in the activity of DPPH in the reaction medium. The antioxidant capacity of a compound is higher as its IC50 is lower.

2.10 STATISTICAL TREATMENT

The results of the dosage of the different phenolic compounds, the antioxidant activity is subjected to the analysis of variance test with a single classification criterion (ANOVA) at the threshold of 5%. The factor in our case is the type of extract. These analyzes are carried out using SPSS 2022 software; we consider that the differences are significant when $P^* < 0.05$, highly significant $P^{**} \leq 0.01$ and very highly significant $P^{***} \leq 0.001$ (Aouadj et al., 2020 a-f ; Aouadj, 2021 ; Aouadj et al., 2022 a-c).

3 RESULTS AND DISCUSSIONS

3.1 EXTRACTION YIELD

The aqueous extracts obtained after drying in a drying oven were weighed to determine the resulting dry weight. The yields were determined in relation to the total weight of the plant material reduced to powder. The infused presents 17.45% of the weight of the dry matter of the leaves from P. lentiscus followed by the macerated with 14.35% and finally the decocted with a yield of 12.75%. The one-way analysis of variance (ANOVA) shows that the difference is significant between the three types of aqueous extracts (p-value = 0,0499) (Table 1) :

Table 1. One-way analysis of variance (extraction yield) (authors, 2024).

ANOVA					
	Sum of squares	df	Mean square	F	p-value
Between Groups	11.420	2	5.710	.	0.0499.
Within Groups	.000	0	.		
Total	11.420	2			

3.2 ANTIOXIDANT ACTIVITY TOTAL PHENOL CONTENT OF THE DIFFERENT AQUEOUS EXTRACTS

The average concentrations of total phenols characterizing the aqueous extracts were evaluated from the regression equation (Y = 0.00219 X – 0.02081) of the calibration range established with gallic acid (0 to 50 µg /mL) and are expressed in mg gallic acid equivalent. We notice from the calculated results that the highest concentration is recorded in the aqueous decoction with the value of 98.23 mg gallic acid equivalents /g followed by that evaluated on the macerated and infused respectively (24.173 and 21.20 mg gallic acid equivalents /g). The one-way analysis of variance (ANOVA) showed good significance for the decoction (p-value = 0.019), this last having the highest level of total phenols compared to the other extracts (Table 2).

Table 2. One-way analysis of variance (Total phenolic content) (authors, 2024).

ANOVA					
	Sum of squares	df	Mean square	F	p-value
Between Groups	33.550	2	15.470	.	0.019.
Within Groups	.000	0	.		
Total	33.550	2			

SS: Sum of squares, df: degrees of freedom, MS : Mean square, F : Observed F-statistic.

3.3 TOTAL FLAVONOID CONTENT OF THE DIFFERENT AQUEOUS EXTRACTS

The quantity of flavonoids in the different aqueous extracts was determined from the calibration curve established from solutions of different concentrations of catechin (Y = 0.0004932 X – 0.00405). The results obtained show that the aqueous macerated is the richest in flavonoids with an average concentration of 91.288 mg catechin equivalents/g of extract followed by the decoction with 50.314 mg catechin equivalents/g of extract and finally the infused with 39.767 mg catechin equivalents/g extract. The one-way analysis of variance (ANOVA) showed good significance for the macerated (p-value = 0.02), this last present the highest level of flavonoids compared to the other extracts (Table 3).

Table 3. One-way analysis of variance (Flavonoid content) (authors, 2024).

ANOVA					
	Sum of squares	df	Mean square	F	p-value
Between Groups	57.45	2	5.710	.	0.02.
Within Groups	.000	0	.		
Total	11.420	2			

3.4 ANTIOXIDANT ACTIVITY: REDUCTION TEST AND PERCENTAGE OF FREE RADICAL INHIBITION

The antioxidant activity of the aqueous extracts (maceration, decoction and infusion) from the leaves of P. lentiscus and the phenolic standard (ascorbic acid) against to the free radical DPPH nm was evaluated by spectrophotometry. The absorbance of DPPH measured at 515 nm shows for all the aqueous extracts and the standard tested a rapid free fall of them as a function of the increase in concentration.

The percentage of stable radical inhibition (DPPH) recorded by the different aqueous extracts and the phenolic standard are shown in figures (2,3,4,5). These results show that the anti-radical effect of aqueous extracts and ascorbic acid is dose-dependent. Indeed, whatever the aqueous extract used, the percentages of inhibition of the stable radical (DPPH) always increase with increasing concentrations. The highest percentages are obtained with the highest concentrations (20 mg/mL for extracts and 5 µg/mL for ascorbic acid): For aqueous extracts of P. lentiscus: the decoction with 100% inhibition for the concentration 10 mg/mL followed by maceration with 100% inhibition for the concentration 20 mg/mL and finally infusion with 82.99% inhibition for the concentration 20 mg/mL. Concerning the phenolic standard: ascorbic acid 100% inhibition for the concentration 5 µg/mL.:

Figure 2: Antioxidant activity (in %) of aqueous extract (decoction) of P. Lentiscus (authors, 2024).

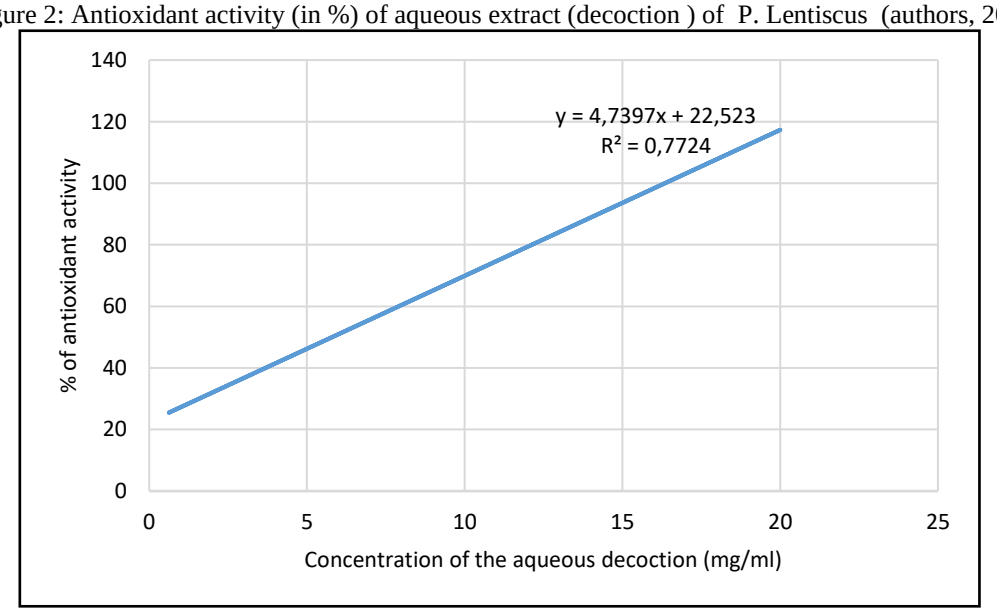


Figure 3: Antioxidant activity (in %) of aqueous extract (maceration) of P. Lentiscus (authors, 2024).

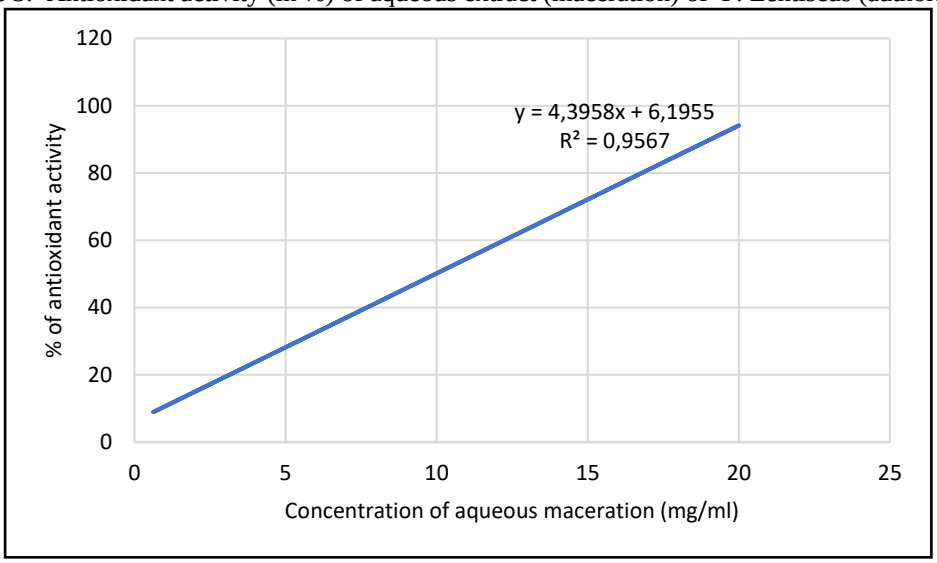


Figure 4: Antioxidant activity (in %) of aqueous extract (infusion) of P. Lentiscus (authors, 2024).

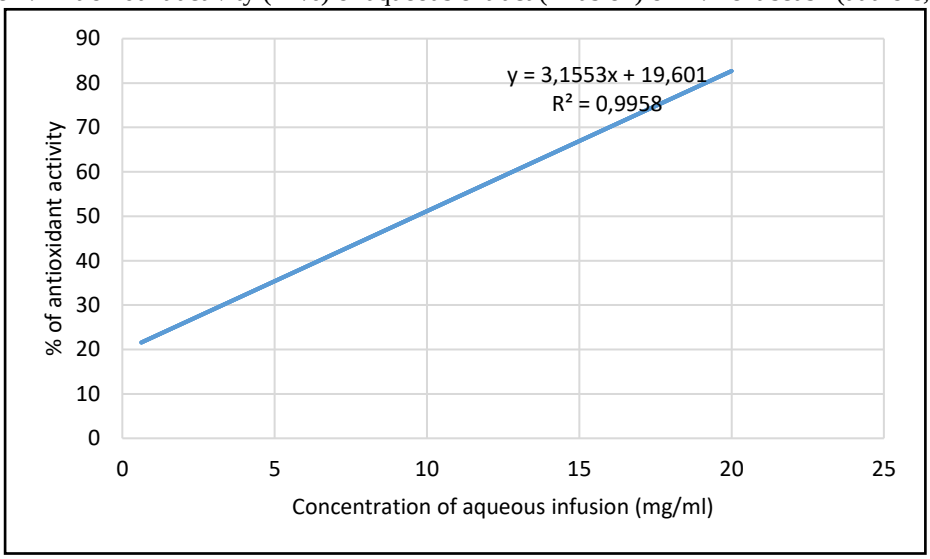
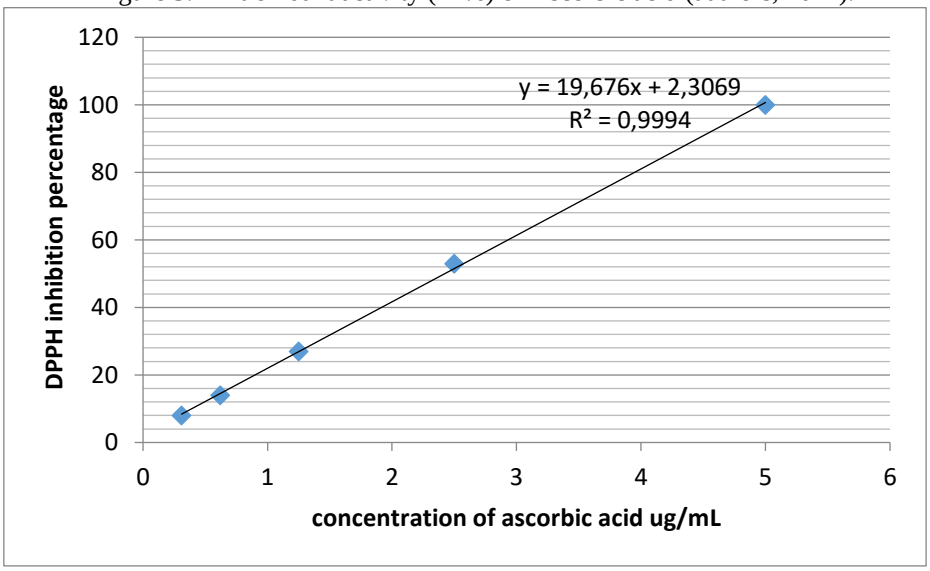
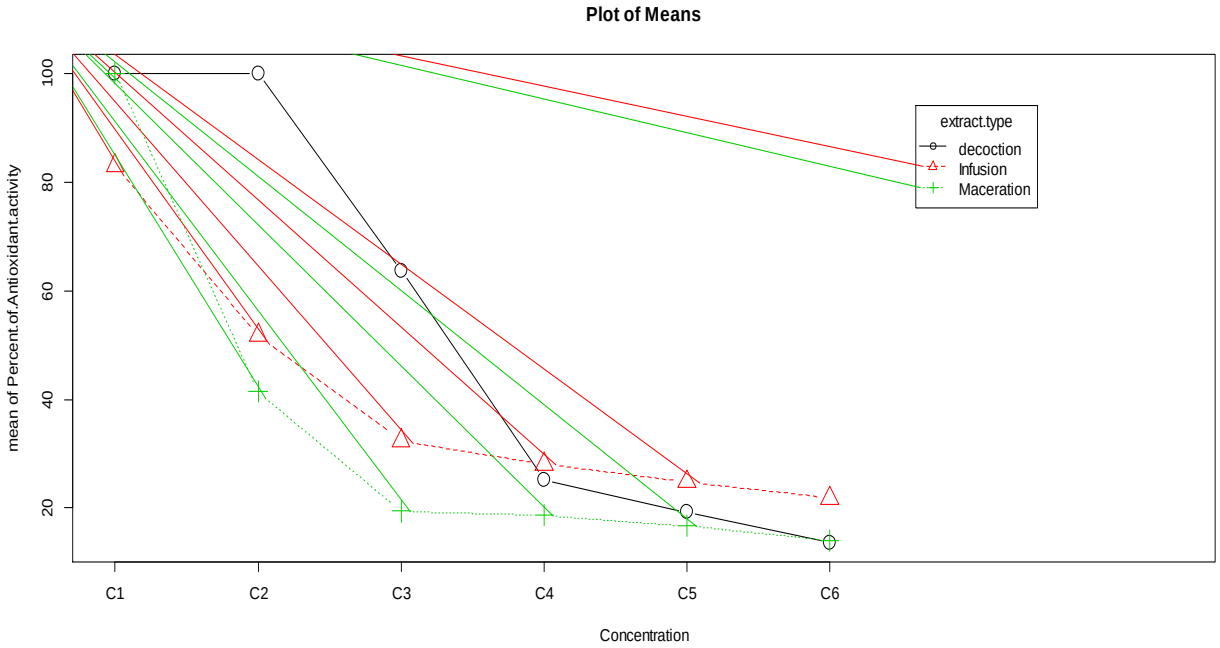


Figure 5: Antioxidant activity (in %) of Ascorbic acid (authors, 2024).



The one-way analysis of variance (ANOVA) confirms the variation in the percentage of antioxidant activity as a function of the aqueous extract and as a function of its concentration (Figure 6).

Figure 6: One-way analysis of variance (% of antioxidant activity) (authors, 2024).



C1 : 20 mg/mL ; C2 : 10 mg/mL ; C3 :5 mg/mL ; C4 : 2,5 mg/mL ; C5 : 1,25 mg/mL ; C 6 : 0,625 mg/mL
Sources: Authors.

The plot of means reveals the difference in antioxidant activity in the both concentrations (20 mg/mL and 10 mg/mL) compared with the three extraction methods. This result is confirmed by the Tukey test (Table 4).

Table 4. Tukey test (% of antioxidant activity depending on extract type and their concentrations) (authors, 2024).

Concentration (mg/mL)	Mean	Groups		
20	94.33	A		
10	64.39333	A	B	
5	38.49		B	C
2.5	23.98		B	C
1.25	20.25667		B	C
0.625	16.482			C
Means that do not share a letter are significantly different.				

3.5 MINIMUM INHIBITORY CONCENTRATION 50 (IC50)

The anti-radical activity of the aqueous extracts and the phenolic standard evaluated by the DPPH test is expressed by the minimum inhibitory concentrations 50 (IC50). The IC50 is inversely related to the antioxidant capacity of the extract tested, because it expresses the quantity of antioxidant necessary to reduce the concentration of the free radical to 50%. The lower the IC50

value, the greater the antioxidant activity. The results mentioned below show that the minimum inhibitory concentrations 50 vary depending on the aqueous extract tested. The IC50 values are: For aqueous extracts: decocted (5.997 mg/mL), infused (9.634 mg/mL) and macerated (9.965 mg/mL). Concerning the phenolic standard: ascorbic acid (2.420 µg/mL).

The One-way analysis of variance (ANOVA) confirms the non-significance of the decocted by the two other extracts in particular the macerated and the decocted this explains that the decocted extract presents the highest antioxidant power when comparing it with the two others (Table 5).

Table 5. The One-way analysis of variance (ANOVA 1) (Minimum inhibitory concentration 50 (IC50) (authors, 2024).

Extract type	Df	SS	MS	F	P-value	Results signification
Maceration	5	0.444467	0.215157	31.0242	0.0057	Very Significant
Decoction	5	0.65523	0.15548	38.0510	0.2140	Not significant
Infusion	5	0.325456	0.167777	33.1000	0.0089	Very significant

It is difficult to compare the results obtained with those in the bibliography because the use of different extraction and quantification methods reduces the reliability of a comparison between studies (Lee et al., 2003 ; Aouadj et al., 2020 a-f). In addition, several factors can influence the content of phenolic compounds because studies have shown that extrinsic factors such as geographic, climatic and environmental factors (Ebrahimi et al., 2008) genetic factors but also the degree of maturation of the plant and the harvest period have a strong influence on the polyphenols content (Aganga & Mosase, 2001 ; Pednault et al., 2001 ; Miliauskas et al., 2004 ; Fiorucci, 2006; Heni et al., 2024).

Therefore, we have contented ourselves with giving examples of some work carried out on the same plant with extraction and dosage methods more or less similar to ours in order to have an idea of the chemical composition of the species against to the phenolic compounds measured. According to Mehenni et al., (2016) the ethanolic extract from the leaves of Pistacia lentiscus L. contains 517.51 mg catechin equivalents/g of extract for polyphenols on the other side the chloroform extract has 1104.6 mg catechin equivalents/g of extract and for the determination of flavonoids the crude extract represents 108.67 mg rutin equivalents/g of extract.

The antioxidant activity of aqueous extracts from P. lentiscus leaves is expressed as inhibitor concentration 50 (IC50). These two parameters are used by several researchers to present the antioxidant activity of different secondary metabolites extracted from various plants (Atoui et al., 2005 ; Wang et al., 2008).

All extracts have anti-radical power against DPPH, the lower the IC50 value, the more the extract is considered a powerful antioxidant (Kadri et al., 2011).

Most studies conducted in this context explain the variations in antioxidant activity depending on the extraction solvents by the polarity of it which influences the extraction yields and consequently the phytochemical composition and the antioxidant potential (Barwick, 1997 ; Kolar et al., 2002 ; Yang et al., 2014). Some authors have suggested that the variation in antioxidant activity depending on the extraction solvents is mainly due to their variabilities in their DPPH absorbance profiles (Shizuka & Kawabata, 2005 ; Sharma & Bhat, 2009).

4 CONCLUSION

The present work aims to study Pistacia lentiscus L. populating Mount Tessala (Western of Algeria) through the quantification of these phenolic compounds (total phenols, flavonoids) and the evaluation of the anti-radical power of aqueous extracts (macerated, decocted and infused) from the leaves.

The quantification of these polyphenols by UV spectrophotometry allowed us to observe that the high concentrations are noted in the decocted aqueous extract for total phenols followed by maceration for flavonoids, thus confirming the variation in concentrations depending on the extract chosen, indicating that mastic, like many other species, is a promising source of compounds phenolics.

The results obtained on the antioxidant activity can contribute to the knowledge of the pharmacological potential of this species, approving its use in traditional medicine while offering modern medicine a potential that can be exploited.

At the end of this study, we hope that this work opens a wide field for the study of this species which deserves to be valued.

In future studies, we will try to develop new natural antibacterial, antifungal agents, essential oil of medicinal plant of the Algerian flora.

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