https://doi.org/10.33472/AFJBS.6.5.2024- 2649-2658

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Assessment of Phytochemical Composition, Antioxidant Properties and Ftir studies of Methanolic and Ethanolic extract of *Pistacia lentiscus* from Algeria Tiaret region

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ArticleInfo

Volume6,Issue5,

Received:18April2024

Accepted:20May 2024

Published:25 May2024

doi:10.33472/AFJBS.6.1.2024 2649-2658

ABSTRACT:

Pistacia lentiscus L. is a shrub from the genus Pistacia, one of the Anacardiaceae families consisting of more than eleven species, widely distributed in the Mediterranean basin.This work was undertaken to enhance the different extracts studies of Pistacia lentiscus as a source of polyphenols and natural antioxidants, as well as the FTIR spectroscopy analysis, which was performed to identify the different function of this species. The screening of Pistacia lentiscus revealed the existence of bioactive compounds: flavonoids, phenols, tannins, anthocyanins and sterols. Pistacia lentiscus demonstrated a strong antioxidant activity for both extracts, with the highest values of total phenolics (4475.2 ±0.56 1.06 mg GAE/100 g) and flavonoids (29.52 ± 0.25 mg QE/100 g) found in the plant methanol extracts.The methanol extracts of the plants revealed highest values of total phenolics (4475.2 ±0.56 1.06 mg GAE/100 g), flavonoids (29.52 ± 0.25 mg QE/100g), the evaluation of antioxidant activities showed a strong antioxidant activity of Pistacia lentiscus for the two extracts.

Keywords: Pistacia Lentiscus, Extracts, Ftir, phytochemical composition.

Introduction

The Algerian forest is part of a Mediterranean complex covering a larger area, characterized by its typical flora, which is an essential element in ecologic, climatic and socio-economic balance of different country's regions (Habib et al., 2020). This flora includes aromatic and medicinal plants, most of which grow wild. Making the most of these plants remains an area of great importance for the country (Beghlal et al., 2016).

Because of the richness and the diversity of its flora, Algeria is a veritable phylogenetic pool, with around 4,000 species and subspecies of vascular plants (Dobignard and Chatelain, 2013). However, little is known about Algeria's medicinal flora, as only 146 of the country's several thousand plant species are listed as medicinal (Baba Aissa, 1999).

Despite advances in pharmacology, the therapeutic use of medicinal plants is still widespread in some countries of the world, particularly developing countries (Hadjadj, et al., 2019; Salmerón-Manzano et al., 2020). In Algeria, this preserved knowledge is passed on from one generation to the next in an empirical manner, guided by tradition. Among the medicinal plants still unexploited, the pistachio tree occupies an important place in the plant cover of the forests in the Tiaret region. Pistacia lentiscus L. is a shrub from the genus Pistacia, one of the Anacardiaceae families consisting of more than eleven species, widely distributed in the Mediterranean basin (Baratto et al., 2003; Azib et al., 2019). P. lentiscus is found throughout Algeria's littoral and thrives in a variety of environments over a range in temperature, precipitation, and sun radiation. The fruit, galls, resin, and leaves of the P. lentiscus tree have been utilized in traditional medicine since the time of the ancient Greeks. These materials have various uses (Trabelsi et al., 2011; Dellai et al., 2013).

This plant is well-known around the world for a number of medicinal qualities, including its anti-inflammatory, antibacterial, and antioxidant activities (Beghlal et al., 2016; Dellai et al., 2013).. Its profusion of flavonoids, phenolic compounds, essential oils and other bioactive ingredients presents an opportunity for the creation of innovative pharmaceutical drugs (Cheraft-Bahloul et al., 2017). Also, the leaves can be used to treat gastro-intestinal and mental disorders as well as to make decoctions and infusions against rheumatism and asthma (Landau et al., 2014; Bozorgi et al., 2013; Azib et al., 2019).

The objective of this study is to evaluate chemical composition and antioxidant effect of and methanolic, ethanolic extracts of the leaves of Pistacia lentiscus.

MaterialandMethods

Plant material preparation

P Leaves. lentiscus were harvested during autumn from the sdama chergui state forest at a place called Ain halouf -Medroussa, region of Tiaret, where the geographical coordinates are: X :331136.816E, Y : 3896012.933N. The leaves were sorted and air-dried at room temperature, protected from light and humidity. Once dried, they were ground and screened using electrical grinder. Resultant powders were analyzed for their chemical and biological aspects.

Phytochemical analysis of Pistacia lentiscus simplesPreparation of extracts

Extraction

Leaves where ground using an electric mill to obtain fine powder. Forty grams of the powder were macerated separately in a solvent [140ml solvent (ethanol, methanol) +60ml distillated water] followed by continuous stirring at room temperature for 24 h. The crude extracts were obtained after dry evaporation in a using rotary vacuum evaporator filtrate.

Solubilization of extracts tested

Residues were collected and used for the experiment. Dry weight of the extract was determined and the extraction yield was calculated using the formula:

$$\text{Yield (\%)} = (\text{weight of dried extract/weight of plant starting material}) \times 100$$

Dry extracts were solubilized in 100% DMSO by diluting 250mg of dry extract in 5ml of solvent.

Phytochemical tests

Phytochemical screening

According to Trease, Evans and Sofowora, qualitative tests were carried out to identify the presence of certain phytochemicals in the plant extract

Determination of total phenolic content

The total phenolic content of the extracts was obtained using the Folin-Ciocalteu method (AL-Farsi et al., 2005; Agbonon and Gbeassor, 2009). 200µl of extract (1mg/ml) was mixed with 1500µl of freshly prepared Folin-Ciocalteu reagent (10-fold dilution). Following a 5-minute reaction, 1500µl of 60% Na_2CO_3 was incubated at room temperature for 90 minutes. Using a spectrophotometer, the volume was measured at 765 nm against a blank.

Determination of total flavonoids content

The $AlCl_3$ colorimetric method (Kim et al., 2003) was used to determine the extracts' total flavonoid concentration. 200µl of extract, 1.2 ml of distilled water and 0.09 ml of a 5% $NaNO_2$ solution were combined. 0.06 ml of a 10% $AlCl_3$ solution was added after five minutes. Following a 5-minute incubation period, 0.6 ml of a 1M Na_2CO_3 solution and 0.75 ml of distilled water were added to the prior combination. Following that, a vortex was used to agitate the mixture. Also, a UV-Vis spectrophotometer was used to detect the solution's absorbance at 510 nm. Using a calibration curve, the results were reported as milligrams of Quercitin Equivalent (QE) per 100 g of dry matter.

Fourier transforms infra-red spectroscopy (FTIR)

This technique is used to obtain the absorption spectrum. The spectral resolution in the number of waves per cm is equal to the reciprocal of the maximum delay (difference of step) in cm. therefore, a resolution of 4 cm-1 will be agreed by a delay of 0.25 cm. These spectra are ended from a sample of dried extract of Pistacia lentiscus. , scattered in a powder of KBr (Potassium bromide), which are modeled in the shape of a fine and transparent pastille and then introduced into the IR spectrophotometer. IR spectra are recorded on a FTIR-8201 PC Spectrometer. The main absorption bands are given in cm^{-1}.

Antioxidant activity

The antioxidant activity of the extracts was obtained by the method based on the reduction of DPPH by antioxidants according to the protocol described by Sanchez (2002). Readings were taken by measuring absorbance at 517 nm. The positive control was represented by a solution of a standard antioxidant and ascorbic acid, with measured absorbance under the same conditions as the samples and for each concentration, the test was repeated 2 times. The results are expressed using the following formula

$$\% \text{ inhibition} = \frac{A\ control\ - A\ sample}{\text{A control}} \times 100$$

Where

$A_{control}$ - absorbance of DPPH solution without extract,

A_{sample} - absorbance of sample with DPPH solution.

Results and Discussion

Plant extract yield

The results (Table 1) show that among the three fractions, the residual Methanolic represents

the highest yield. Thus, the yields of plant extracts obtained for the two types of extracts: hydro-ethanolic and hydro-methanolic are 48.85% and 49.52%, respectively. The extraction yield is the ratio of the quantity of natural substances extracted by the extractive action of a solvent to the quantity of these substances contained in the plant material. It depends on several parameters such the solvent, pH, temperature, extraction time, and the sample composition (Quy Diem Do et al., 2014).

Table 1. The yield of the vegetable extracts of the plants used.

Extract type	**Methanolic**	**Ethanolic**
Yield %	48.85%	49.52%

Screening for phytochemicals

The phytochemical test results demonstrate the extract's richness in several active ingredients. The chemical contents of Pistacia lentiscus include poly- phenols, tannins, flavonoids, and anthocyanin. The results are expressed in the Table 02

Table 2. Phytochemical constituents of Pistacia lentiscus extracts

	***Pistacia lentiscus* constituents**					
	Flavonoids	**Tannins**	**Anthocyanin**	**Sterolset triterpene**	**Phenols**	**Saponosides**
Methanolic extracts	+	+	+	_	+	_
Ethanolic extracts	+	+	+	+	+	-

Total phenol, flavonoid of different Pistacia lentiscus extracts

The total phenol and flavonoid content were calculated using the following equations based on the calibration curve: (y=0,0056x+1,0519) for total phenol and (y=0.0692x+0,0188) for total flavonoid in comparison with standards equivalent: Gallic acid (mg GAE/100g) and Quercetin (mg QE/100g), respectively.

Phenolic compounds are usually found in plant components such leaves, stems and barks, flowers, and fruits, The polyphenols are confirmed as a natural antioxidant (Namukobe et al; 2021). The results are represented in Table3, it can be seen that the plant extracts studied are

rich in polyphenols but with different amounts. In this study, the methanolic extract obviously has more fractions, ethanol. According to Barbouchi et al, 2020, Pistacia lentiscus leaves are extremely rich in phenolic compounds (varied from 345,95 ± 1,17 to 67,83 ± 0,36 mg of GAE/g of extract) .

Numerous studies have shown that the Pistacia lentiscus L. plant has an abundance of phenolic compounds, with this abundance increasing with the polarity of the extraction solvents used. (Bampouli et al.,2014; Botsaris et al., 2015; Zitouni et al., 2016).

Table 3. Total phenolic contents and total flavonoids content of different Pistacia lentiscus extracts.

Pistacia lentiscus	**Total phenolic content (mgGAE/100g)**	**Total flavonoid content (mg QE/100g)**
Methanol extract	4475.2±0.56	29.52± 0.25
Ethanol extract	**1393.2±0.06**	**22 ± 0.95**

Fourier transform infrared spectroscopy (FTIR) compounds

The FTIR analysis extracts leaves proved the presence of aromatic rings, phenols alkenes, aliphatic, alcohols, ethers, esters, nitro compounds and hydrogen bonded alcohols. (Fig. 1).

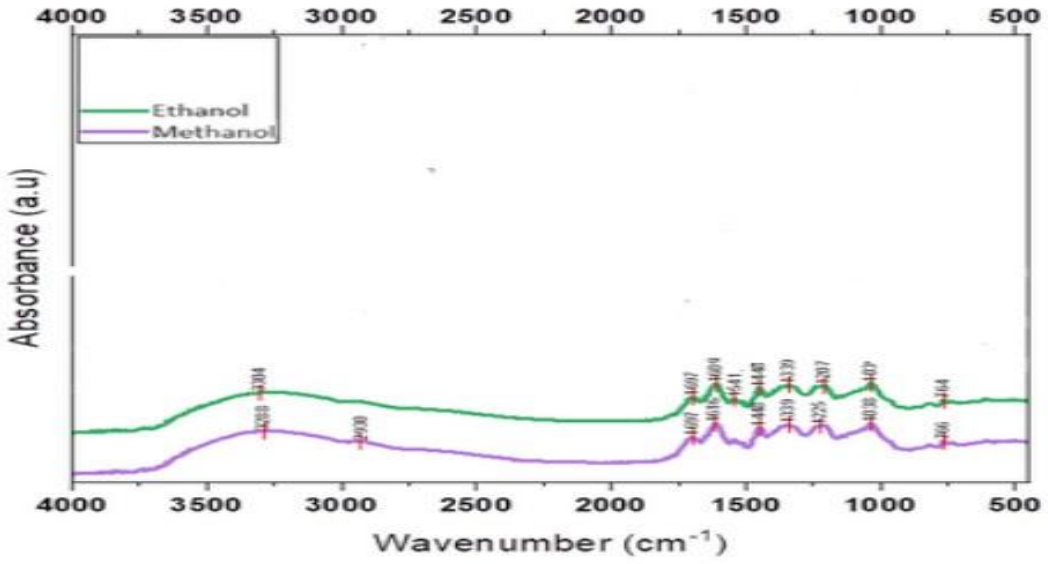

Fig. 1. The infrared spectrum of *Pistacia lentiscus* methanolic and ethanolic extracts.

Indeed, the bands around 766-764 and 1207-1222 cm^{-1} are assigned to the C-H link (aliphatic function); the bands around 1038 and 1036 correspond to the C=O bond (alohol, ether or ester functions); the bands 1339 are associated to the amine fuctions , the narrow bands around 16016 cm^{-1} correspond to theC=C (alkene, conjugated double bond); the bands 1697 cm^{-1} are assigned to the Ketone. And finally, the wide bands around 3304 and 3288 cm^{-1} are associated with the elongation vibration of the OH (alcohol or phenol function) bond.

Phytochemical analysis of the ethanolic and the methanolic extracts of pistacia lentiscus by Fourier transform infra red spectroscopy, or FTIR spectroscopy, revealed the presence of different families of chemical compounds (Table 4.) such as phenols, (cyclic compounds) (OH , C-H ,C-O ether o, C-N amine , C-O ether ans ester, C=C and C=O).

Table 04: Results of Phytochemical analysis of Pistacia lentiscus by FTIR study

Wavenumber (cm^{-1})		**Band assignment**
Ethanol	**Methanol**	
3304	3288	O-H stretching (alcohol or phenol)
	2930	C-H stretching (aliphatic)
1697	1697	C=O stretching in unconjugated ketone
1609	1616	C=C stretching (alkene, conjugated double bond)
1541		C=C stretching (alkene, conjugated double bond)
1448	1448	C-H bending (aliphatic)
1339	1339	C-N bending (amine)
1207	1225	C-H bending (aliphatic)
1036	1038	C-O stretching (alcohol, ether, or ester)
764	766	C-H bending (aliphatic)

Antioxidant activity:

The radical DPPH is generally one of the most used compounds for the rapid and the direct evaluation of antioxidant activity due to its stability in radical form and the simplicity of the analysis (Gulcin, et al., 2004). From the IP inhibitory power results, it can be seen that the two extracts show higher antioxidant activity. The results for the different extracts at a

concentration of 1mg/ml, estimate that the methanolic extract has an inhibitory power of 90.39/ and the ethanolic has an antioxidant power of 65.40%.

Barbouchi et al., (2020) investigated the DPPH scavenging activity of different extracts of Pistacia lentiscus collected from two regions in Morocco. They reported IC50 values of 1.13 and 0.57 mg/ml for the methanolic leaf extracts. Dahmoune et al., (2015) used three techniques for extraction (ultrasound-assisted extraction, accelerated solvent extraction and conventional solvent extraction) and obtained IC50 values between 18.74 - 32.77 µg/mL. Our results are in good agreement with those of Yosr et al., (2018), who tested Pistacia lentiscus samples of different maturation stages and found mean values ranging between 4.9- 6.4 µg/mL. The same trend was observed in the study by Gardeli et al., (2008), who evaluated the DPPH radical scavenging activity in three different stages (February, May and August). They obtained IC50 values ranging from 5.09 - 11.0 mg/L. They concluded that the highest antioxidant activities were observed in samples from the full flowering stage.

Conclusion

In this study, this plant was investigated because it offers interesting phytochemicals that can be used in in vitro tests to measure the biological activity of Pistacia lentiscus plants that are grown in Algeria. The aerial part phytochemical compounds of Pistacia lentiscus have significant antioxidant. As a result, Pistacia lentiscus may provide excellent sources of new bioactive natural products that can serve as new pharmaceutical agents to address unmet therapeutic needs. However, further research is needed, especially in vivo antioxidant, anti-inflammatory.

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