EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2023, 24(7), 172–177
https://doi.org/10.12912/27197050/169935
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Received: 2023.07.01
Accepted: 2023.07.24
Published: 2023.08.15

Phytochemical, Anti-Inflammatory and Antioxidant Activities of *Pistacia lentiscus* L. Leaves from Ajdir, Al Hoceima Province, Morocco

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ABSTRACT

Pistacia lentiscus L. (PL) is a shrub belonging to the Anacardiaceae family, used in traditional medicine to treat various disorders in the Commune of Ajdir from Al Hociema province. The conducted study focused on determination of the polyphenols and flavonoids contents by spectrophotometric and in vitro evaluation of the antioxidant and anti-inflammatory capacity. Determining total polyphenols shows the aqueous extract with a higher concentration of 125.04±0.01 mg EAG/g ES, followed by the ethanolic extract of 108.16±0.02 mg EAG/g ES. Determination of flavonoids revealed that the hexanoic extract contains a maximum of flavonoids with a level of 90.60±0.01 mg EQ/g ES. The antioxidant activity of different extracts was determined through two methods: DPPH and FRAP. Aqueous and ethanolic extracts showed a high antioxidant capacity. Further, regarding anti-inflammatory activity, the ethanolic extract has good activity inhibition (92.65±0.67) followed by aqueous extract (94±0.29) at 1000 µg/mL concentration. This study found that the ethanolic extracts from PL leaves are a powerful natural antioxidant and effective anti-inflammatory agent. The results indicate the extract's effectiveness and highlight the importance of medicinal plants from the Commune of Ajdir.

Keywords: *Pistacia lentiscus*, antioxidant activity, anti-inflammatory, polyphenols, medicinal plants, Al Hoceima, phytochemical.

INTRODUCTION

The Mediterranean Region is known for its high richness of plant species, especially medicinal and aromatic plants (Hadjichambis et al., 2008; Leonti et al., 2010; Chaabani et al., 2020; Nocentini et al., 2022). Among them, one can find *Pistacia lentiscus* L. (PL), known as a mastic tree native to the Mediterranean basin of North Africa, of which the region of Al Hoceima is part (Dragović et al., 2020; El Bishbishy et al., 2020; Milia et al., 2020). *Pistacia lentiscus L*. is part of the Anacardiaceae family; it can reach up to 5 meters in height, it is evergreen, oval, and shiny (Ladio et al., 2007; Trabelsi et al., 2012). Since antiquity, the Greeks have used the mastic tree to treat urinary tract diseases, using its antiseptic and anti-inflammatory effects (Andreadou et al., 2016; Milia et al., 2021). In turn, the Romans used it in food as a flavoring and also for its medicinal properties (Emna Chaabani,. 2019). *Pistacia lentiscus* L. is still used for its medicinal and aromatic properties. Its resin is known as mastic used in cooking for flavoring as well as in the manufacture of cosmetics, food additives, and perfumes (Pachi et al., 2020). PL leaves have been traditionally used for their medicinal properties to treat various infections, including respiratory, gastrointestinal diseases, as well as menstrual plus joint pain (Dellai et al., 2013; Vogiatzoglou et al., 2015). Many studies have demonstrated that leaves and fruits contain phenolic compounds and flavonoids (Bampouli et al., 2015; Ait Mohand et al., 2020); this explains the elevated antioxidant

capacity, which helps protects cells from damage caused by free radicals. It also has anti-inflammatory action by inhibiting the production of inflammatory cytokines and reducing the expression of inflammatory genes (Aranega and Boulaiz, 2005; Boutemine et al., 2018; Milia et al., 2021). To recapitulate, the antioxidant and anti-inflammatory properties of PL are promising. However, the efficacy of these activities varies from area to area due to attitude, climate, and human activity. There needs to be more studies on the traditional medicinal plants in the commune of Ajdir. A previous study on traditional medicinal plants in this region was conducted in the Parc National d'Al Hoceima. Nevertheless, it did not investigate the anti-inflammatory properties of these plants. The present study investigated the activity of the antioxidant and anti-inflammatory properties in various solvents polarity on PL cultivated in the commune of Ajdir to compare them with other studies in different regions. In addition, it highlighted the importance and efficacy of medicinal plants from the commune of Ajdir.

MATERIALS AND METHODS

Plant material

PL was collected in June from the commune of Ajdir of Al Hoceima province (35,21023° N, 3,91763° O). The leaves of PL were dried in the dark for a week. The research unit of the Faculty of Science and Technology of Al Hoceima identified the species where a voucher specimen was placed in the herbarium.

Extraction procedure

The leaves of PL were crushed to obtain a powder to extract the natural substances using the Soxhlet apparatus. Then, 30 g of dried leaf powder underwent extraction with 200 ml of different solvents were used with increasing polarity: hexan (EH); ethyl acetate (EA), absolute ethanol (EE), water (EQ).

Determination of total polyphenol (PC) and flavonoid content (FC)

The methods to quantify the level of FC and PC were modified from those used in previous research (Chen et al., 2021; P.H. Huang et al., 2022). The PC was measured using Folin-Ciocalteau reagent (FCR) diluted ten times with distilled water. 200 µl of PL extract solution was mixed with FCR. The mixture is agitated and incubated for 4 min. Then, 0.800 µl of 7.5% sodium bicarbonate solution was added. The solution was incubated for two hours. The absorbance solution was measured at 760 nm. The regression equation was performed by gallic acid at different concentrations under the same operating conditions as that of samples to calculate the PC. The PC is reported in mg gallic acid equivalent/g MS. To determine the FC, 1 ml of extract solution was added to 1 ml of $AlCl_3$ (2%), then agitated and incubated for 30 min (Djeridane et al., 2006). The absorbance at 430 nm was used for the solution measurement. The FC was calculated as the same sample conditions using regression equation applied by quercetin at various concentrations.

Evaluation of in vitro antioxidant capacity

DPPH method

The DPPH assay was measured via the method described by (Alam et al., 2013) with minor modifications. Various sample concentrations (0.2 ml) were mixed with 1.8 ml DPPH solution (0.5 mM). The samples were vigorously mixed and then incubated for 30 minutes in a dark area. The inhibition percentage of DPPH was measured as follows:

$$(AC - AS)/AC \times 100 \quad (1)$$

where: AC – absorbance of control, AS – absorbance of sample.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined with modification according to (H. Huang et al., 2014). This test includes adding 2.5 ml of sodium phosphate buffer (PBS) and 1% of $K_3Fe(CN)_6$ to the extracts and gallic acid at different concentrations. The mixture was incubated at 50°C in a water bath for 30 min, then 2.5 ml TCA (10%), distilled water, and 0.5 ml $FeCl_3$ were added to the 2.5 ml mixture. The mixture's absorbance was measured at 700 nm against a blank.

Anti-inflammatory activity

The anti-inflammatory activity of PL leaves extracts was determined by the denaturation method of bovine serum albumin (BSA) with

modifications. For this purpose, 500 µl of extracts with different concentrations and standard (Voltarene 75 mg) with 500 µl of BSA solution (0.2%) prepared with phosphate-buffered saline (PBS) at a pH of 7.2 The mixture was incubated at 37°C for 15 min and then at 72°C in a marine bath for a duration of 5 min. Once the tubes were cooled, the turbidity of the samples was measured at 660 nm using a spectrophotometer. The following equation was used to determine the proportion of protein denaturation.(Lekouaghet et al., 2020).

$$PI = (1 - AS/AC)\cdot 100\% \quad (2)$$

where: PI – percentage of inhibition, AS – absorbance of sample, AC – absorbance of control.

RESULTS AND DISCUSSION

Total polyphenol (PC) and flavonoid content (FC)

The results of polyphenol determination using folin reagent revealed that PL leaves have high levels of PC ranged between 125.02±0.04 to 30.94±0.11 mg of GAE/g. In turn, FC is from 90.60±0.01 to 19.90±0.01 as described in Table 1.

The results show that PC varies according to the extraction solvent the PC of which decreased in the following order EQPL> EEPL > EAPL > EHPL, while FC EHPL> EQPL > EAPL > EEPL. The table reveals that the extracts containing more polyphenol are EQPL as well as EEPL due to their excellent polarities and solubilities of solvents (Barbouchi et al., 2020). In comparison, the low polarities solvents such as hexane and ethyl acetate were not effective in extracting phenolic compounds, which explains low PC amount of EAPL and EHPL compared to EQPL and EEPL with high polar solvents. These results are similar to other studies, indicating both aqueous and ethanolic extracts are rich in phenolic compounds (Salhi et al., 2019). Several studies have shown that the amount of polyphenols increases as the polarity of solvents increases, which was found in the obtained results (Galanakis et al., 2013; Zitouni et al., 2016). Concerning the results of FC, the table shows that hexane contains the highest flavonoid content compared to the other solvents tested (EHPL = 90.60±0.01), followed by aqueous extracts (EQPL = 48.69±0.01). In contrast, the ethanolic extract had the lowest content (EEPL = 19.90±0.01). This variation in FC is due to the polarity of solvents (Barbouchi et al., 2019).

Evaluation of in vitro antioxidant capacity

The antioxidant capacity of the various fractions of PL was evaluated using the DPPH and FRAP assays. Table 2 reported results using the IC50 value, which represent the quantity of each extract required to inhibit free radicals at 50%. However, the lower the IC50 value, the more potent the extract inhibits free radicals. Table 2 shows a low IC50 value for EEPL (IC50 = 10.84±0.09 µg/mL) and EQPL (IC50 = 15.85±0.01 ug/mL) extracts in the DPPH assay, as well as in the FRAP assay where EEPL (IC50 = 5.32±0.43 µg/mL) and EQPL (IC50 = 5.37±0.22 µg/mL) also have a low IC50 value signifying the high antioxidant activity of these two PL extracts, which is close to acid ascorbic standard. These results could be attributed to the high polyphenol content of extracts, potent antioxidants that can block the formation of free radicals and counteract the oxidation of macromolecules**.** Similarly, other studies reveal

Table 1. Phenolic compounds of PL leaves at various fractions

Extrats/Fraction	TPC	TFC
EHPL	30.94±0.11	90.60±0.01
EAPL	70.81±0.01	39.52±0.01
EEPL	108.16±0.02	19.90±0.01
EQPL	125.02±0.04	48.69±0.01

Note: the results are presented as mean ± S.D. (n=3), FC – total flavonoids (mg QE g^{-1} powder); hexan extract (EHPL); ethyl acetate extract (EAPL), absolute ethanol extract (EEPL), aqueous extract (EQPL).

Table 2. Antioxidant activity of *PL* leave*s* extract*s* by DPPH, FRAP assay

Extrats/Fraction	DPPH assay	FRAP assay
	IC50 µg/mL	IC50 µg/mL
EHPL	79.19± 0.46	18.52± 0.17
EAPL	134.5± 0.39	14.88± 0.26
EEPL	10.84± 0.09	5.32± 0.43
EQPL	15.85± 0.01	5.37± 0.22
Acid ascorbic	8.58± 0.03	4.310 ± 0.1
BHA	14.37± 0.15	NT

Note: the values presented are expressed as mean ± SD (n=3), BHA: Butylated hydroxytanisole, NT: not tested

Table 3. Inhibition percentage of PL leaves extracts on albumin denaturation

Concentration ug/ml	%Inhibition				
	EHPL	EAPL	EEPL	EQPL	(Voltarene 75 mg)
1000	87.95±0.9	47.27±0.92	94±0.29	92.65±0.67	94.83±0.54
600	75.34±0.44	40.20±0.56	93.09±0.89	90.79±0.76	93.97±0.64
300	62.30±0.78	25.64±0.37	91.87±0.27	90±0.70	92.71±0.67
100	32.07±0.97	29.26±0.90	90.88±0.28	89.09±0.63	91.27±0.57
80	28.71±0.26	27.5±0.35	88.56±0.11	88.56±0.39	90.44±0.19

Note: the values presented are expressed as mean ± SD (n=3).

that polar extracts of PL had a high antioxidant capacity (Boucheffa et al., 2021; Zitouni et al., 2016). Additionally, it was noted that the antioxidant test results strongly correlate with PC (Barbouchi et al., 2020).

Anti-inflammatory activity

It was estimated that the anti-inflammatory activity of different PL extracts were evaluated using bovine albumin denaturation (BSA) method. Table 3 summarizes the inhibition rates of different PL extracts and Voltarene as standard over the entire concentration range from 80 to 1000 µg/mL. EEPL showed the highest inhibition rate of BSA denaturation at 94±0.29, followed by EQPL at 92.65±0.67 (tested at 1000 µg/ml). These values are close to Voltarene (75 mg), an anti-inflammatory drug used as a standard. This beneficial effect can be attributed to the antioxidant compounds contained in PL extracts. These antioxidants protect cells from free radical damage leading to inflammation, which antioxidants can minimize (Arulselvan et al., 2016; Sehaki et al., 2023).

CONCLUSIONS

In summary, the study objectives were to use the BSA denaturation assay and assess the anti-inflammatory activity of PL. The results obtained indicate that PL has very high anti-inflammatory activity. However, additional study is required to confirm results and investigate the specific mechanisms of action. In addition, the conducted study was built to use BSA to investigate; this plant is an important finding, as it suggests that *Pistacia lentiscus* L. may have different properties in this and other areas. Furthermore, the study revealed that ethanol and aqueous extracts have equally high antioxidant activity due to their rich polyphenol content. The obtained findings in the commune of Ajdir demonstrate the importance of traditional plants and the need for further research into their potential therapeutic applications.

Acknowledgments

The authors thank Hassan Amhamdi, Omar Benamari, Yahya El-Mernissi, Amin Salhi, Mhamed Ahari, Soufiane El Barkany for their helpful guidance and support throughout this research project. Yahya El hammoudani, Kaoutar Jellouli, Sanae Jahjah and karima ait alla were not directly involved in the research, but their advice and support were invaluable.

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