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Assessments of Antibacterial and Antioxidant Properties in the Methanolic and Aqueous Leaf Extracts of *Pistacia lentiscus* against Different Antibiotic Resistance Pathogenic Bacteria

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Abstract

The current study was carried out to determine the bioactivity of *P. lentiscus* leaf extracts as potential antibacterial and antioxidant properties. The plant extracts were examined for antibacterial activity against antibiotic-resistant *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* using the agar well method (according to the guidelines of Clinical and Laboratory Standard Institute). The antioxidant potential of 3 plant leaf extracts was determined by their ability to convert Fe^{3+} to Fe^{2+} and scavenge the DPPH free radical. At all concentrations studied, the methanolic leaf extract had higher total phenolic and flavonoid content, as well as stronger antioxidant and antibacterial inhibitory activity compared to aqueous extract. Our findings with *P. aeruginosa* were especially interesting, because this bacterium was inhibited by methanol extract than that of the reference antibiotics. The results also demonstrated a link between DPPH radical scavenging ability, reducing power, and total phenolic and flavonoid content of plant extracts ($r > 0.97$, $R^2 > 0.95$, $P = 0.01$). As a result, the methanolic leaf extract of the chosen plant might be employed as an effective antioxidant and antibacterial agent for the treatment of a variety of morbidities.

Keywords

Antioxidant Activity, Polyphenols, Methanolic Leaf Extracts, DPPH, Reducing Power, *Pistacia lentiscus*

1. Introduction

Despite the recent development of powerful antimicrobial agents, antibiotic resistance has elevated dramatically [1] [2] [3] [4]. Antibiotic-resistant (ABR) pathogens such as *Staphylococcus aureus* [5], *Staphylococcus aureus* [6] [7], *Pseudomonas aeruginosa* [8] [9], and *Proteus mirabilis* [10] are particularly noteworthy as a highly increasing number of nosocomial pathogens (nosocomial infections) are receiving resistance to conventional antibiotics. Ultimately, the emergence of ABR bacteria is taken into consideration to be the most important reason of death worldwide [11]. As a result, novel healing techniques are urgently needed to eliminate pathogenic infectious agents. The medicinal properties of plants are a vast and unexplored source and offer limitless opportunities for new substances for the prevention and treatment of infectious diseases.

Biologically active molecules of plant origin, primarily polyphenols, including flavonoids and phenolic acids, are important sources of natural antimicrobial agents against attackers, particularly microorganisms [12] [13] [14] [15] [16]. Polyphenols are the largest class of bioactive compounds in plants and are formed as secondary metabolites with protecting features towards pathogenic microorganisms [17]. Antioxidants such as flavonoids, phenolic acids and vitamin C are important for inhibiting the formation of various oxidative stressors *in vitro* and *in vivo* due to their capacity to get rid of scavenge reactive oxygen species (ROS) and free radicals [18]-[23].

Pistacia lentiscus (*P. lentiscus*) is an Anacardiaceae, which broadly distributed in the Mediterranean regions [24] [25] [26] [27]. In conventional medicine, *P. lentiscus* is utilized to treat infectious diseases caused by bacteria and other harmful microorganisms [28]. In addition, *P. lentiscus* consists of several bioactive chemical components. Among these, flavonoids and phenolic acids, which have robust antioxidant capacity [29] [30] in addition to immunomodulatory, anti-inflammatory [31] [32] [33] [34], hepatoprotective, and antibacterial effects, were the most abundant compounds in the leaves of *P. lentiscus* [35] [36] [37] [38].

Consequently, characterization of bioactive compounds in *P. lentiscus* leaf extracts is important to meet the growing demand for plant-based drugs. Although many recent studies have been carried out on medicinal plants for their biological properties as natural products and preventative factors against infectious diseases, the search for new and exciting natural compounds still needs further effort as the rapid emergence of bacterial drug resistance. Hence, the aim of the current study was undertaken to evaluate the efficacy of methanolic and aqueous leaf extracts of *P. lentiscus* collected from the region of Al-abyar forest (Libya) as potential antibacterial and antioxidant agents against antibiotic-resistant pathogenic bacteria.

2. Materials and Methods

2.1. Chemicals and Reagents

1,1-Diphenylpicrylhydrazyl ($DPPH^{\cdot}$) was purchased from Sigma Co. (St. Louis, MO, USA) and methanol was supplied from Merck (Darmstadt, Germany). All

other chemicals and reagents were obtained from the biochemistry laboratory of chemistry department, university of Benghazi.

2.2. Plant Material

The leaves of *P. lentiscus* were collected from Al-abyar forest, roughly 60 km to the east of the city of Benghazi, Libya. The plant specimen was identified in the herbarium of Botany department (Faculty of Science, University of Benghazi).

2.3. Preparation of Plant Extracts

Two polar solvents (methanol and aqueous solution) had been selected to extract the chemical constituents of the studied plants. Before extraction, the leaves had been very well washed with tap water and rinsed with distilled water, after which air-dried at room temperature (2527˚C) under shade. The methanol extraction procedure was performed according to Vu *et al.* [39] with minor modifications. 10 g of air-dried plant powder was extracted with 100 ml of methanol for 48 h at room temperature (25˚C). The extracted suspensions were filtered with Whatman No.1 filter paper (Whatman Ltd., England). The methanol was dried using rotary evaporator at a temperature not exceeding 40˚C. The methanol was dried in a rotary evaporator at temperatures no higher than 40˚C. The dry extract was weighed, and the extract concentration was calculated before being stored in a sterile bottle and kept in the refrigerator at 4˚C until use. For aqueous extraction, 25 g of plant powder was mixed with 250 mL of distilled water using a Soxhlet apparatus and filtered through Whatman No.1 filter paper. A 100˚C Rotary evaporator was used to dry the aqueous extract. The dried extract was kept in a sterile bottle at −4˚C until further use [40].

2.4. Polyphenols Analysis of the Extracts

2.4.1. Determination of Total Flavonoid Content (TFC)

Chang *et al.* [41] have used an aluminum chloride colorimetric approach to examine the flavonoid content material of each extract. After 30 minutes of incubation at room temperature, the leaf extract turned into blended with 0.1 ml of 10% aluminum chloride, 0.1 ml M potassium acetate, and 2.8 ml of distilled water in a quantity of 2 ml at diverse concentrations "100, 200, 300, 400, 500 µg/ml." The absorbance of the response combination turned into measured at 415 nm the use of a UV-seen spectrophotometer. Total flavonoid content was estimated as µg of quercetin equivalents/ml of *P. lentiscus* extract.

2.4.2. Determination of Total Phenolic Content (TPC)

The total phenolic content of the extracts was tested by a colorimetric method based on the Folin-Ciocalteu (FC) reagent [42]. 0.05 ml of Folin-Ciocalteu's reagent was mixed with 0.05 ml of different concentrations "100, 200, 300, 400 and 500 µg/ml". The mixture was then adjusted to 1 ml using 0.4 ml of distilled water and 0.5 mL of 15% sodium. carbonate solution. After allowing the reaction to stand for 10 minutes, the absorbance was measured using a visible UV spectro-

photometer at 725 nm. The total content of phenolic component was expressed in µg pyrogallol equivalent/ml of *P. lentiscus* extract. The obtained results were estimated in triplicate and presented as mean values with standard deviations (SD).

2.5. *In Vitro* Antioxidant Activity

2.5.1. Reducing Power Assay (RPA)

The reduced power was calculated on the basis of the Ara and Nur method [43]. 2 ml of leaf extract in various concentrations (100, 200, 300, 400, and 500 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide. The mixtures were then incubated for 20 minutes in a 50˚C water bath before being centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant was mixed with 2.5 ml of purified water and 1 ml of $FeCl_3$, which reacts with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), and then with ferric chloride to give an iron-metal Complex with maximum homogenization at 700 nm using a UVV-capable spectrophotometer. Evaluation was done with regard to stander calibration curve of ascorbic acid (Vitamin C). Vitamin C was utilized for comparison.

$$\text{Potassium ferricyanide} + \text{ferric chloride} \xrightarrow{\text{antioxidant}} \text{potassium ferrocyanide} + \text{ferrous chloride}$$

2.5.2. DPPH Radical Scavenging Activity (RSA)

The antioxidant capacity of the leaf extracts was tested using the DPPH (diphenylpicrylhydrazyl) method as described via Rao *et al.* [44]. A reaction mixture containing 2 ml of each extract at different concentrations "100, 200, 300, 400, 500 g/ml" and 2 ml of DPPH (0.2 mM) was vigorously shaken and incubated at room temperature for 30 minutes in the dark. When DPPH reacted with a hydrogen-donating antioxidant chemical, it was reduced, resulting in a decrease in absorbance at 517 nm measured with a UV-visible spectrophotometer and the mean values of three experiments. The sample concentration was plotted against the proportion of residual DPPH. A lower value indicates greater antioxidant capacity. The scavenging activities were estimated as a percentage inhibition of DPPH and calculated using the formula:

% inhibition of DPPH "RSA" = [Abs. of Control − Abs. of Sample/Abs. of Control] × 100

where Abs control is the absorbance of DPPH without extract and Abs sample is the absorbance of DPPH along with different concentrations of extracts. The IC_{50} value represents the concentration of antioxidant compounds that reduce free radicals by 50%, was calculated using the percentage DPPH inhibitory activity against the different concentrations of extracts.

2.6. Antibacterial Activity of the Extracts

2.6.1. Microorganisms

The antibacterial activity was assessed using Gram positive bacteria: *Staphylo-*

coccus aureus (*S. aureus*) *Staphylococcus haemolyticus* (*S. haemolyticus*), and Gram negative bacteria: *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Proteus mirabilis* (*P. mirabilis*), which are known to be antibiotic-resistant bacteria. These bacterial species were obtained from the microbiology laboratory of Benghazi children's hospital. The microorganisms were isolated and identified using standard methods at El-Jala Teaching Hospital, and their identification was confirmed using phoenix.

2.6.2. Evaluation of Antibacterial Activity of the Extracts

The antibacterial effect of the leaf extract was examined using the agar well diffusion method [45]. Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs across the entire surface of the plate after immersing 100 µg bacterial suspensions on plates (overnight cultures grown at 37˚C on nutrient agar and adjusted to 0.5 McFarland in sterile saline). A well was created in the agar using a sterile cork borer measuring 9 mm in diameter. The plant extract was added to the wells at various quantities (25, 50, 75, and 100 percent), and the plates were incubated at 37˚C for 24 hours. Levofloxacin (5 µg) and ampicillin were used as positive controls (10 µg). Negative controls included pure methanol solvents and watery solutions. Under suitable conditions, the experiment was repeated three times for each concentration. The antibacterial activity against the microorganisms is indicated by the zone of inhibition surrounding the wells. The diameter of the zone of inhibition was measured with a ruler, and isolates and antibiotic discs were classified as susceptible, intermediate, or resistant using the Clinical and Laboratory Standards Institute (CLSI) breakpoint system [46].

2.7. Statistical Analysis

GraphPad prism version 8.4.3 (686) was employed for statistical analysis and graphical representations of results. One-way analysis of variance (ANOVA) with Tukey's test were used for statistical comparisons and all tests were considered statistically significant at $P < 0.05$. All results were expressed as mean ± standard deviation (SD) in triplicate. Data was evaluated using Pearson's correlation coefficients (r) and coefficient of determination (R^2) to identify relationships between antioxidant activity, total phenolic and flavonoid contents.

3. Results

3.1. Polyphenols Composition of the Plant Extracts

The results exhibited that the total phenolic and flavonoid content in the methanolic extract of *P. Lentiscus* was higher than that of aqueous extract (**Table 1**). Furthermore, in both extracts, the content of flavonoid and phenolic constituents increased with increasing concentration.

3.2. Antibacterial Activity of the Plant Extracts

The results of screening plant extracts for antibacterial activity are summarized in **Table 2** and **Figure 1(a)** and **Figure 1(b)**. In relation to the solvent used,

Table 1. Total flavonoid content (TFC), total phenolic content (TPC), % inhibition of DPPH radical scavenging, reducing power, the values of IC_{50} in methanol and aqueous leaf extracts of *Pistacia lentiscus* compared to the standard vitamin C at various concentrations.

Solvent	Concentration (µg/ml)	Total flavonoid content (TFC)	Total phenolic content (TPC)	DPPH scavenging activity (%)	Reducing power activity	IC_{50} (µg/ml)
Methanol	100	0.273 ± 0.022	0.352 ± 0.046	32.5	0.398 ± 0.033	189.62
	200	0.482 ± 0.074	0.612 ± 0.062	49.4	0.605 ± 0.062	
	300	0.712 ± 0.083	0.843 ± 0.052	68.4	0.801 ± 0.043	
	400	1.021 ± 0.053	1.19 ± 0.034	85.0	0.971 ± 0.066	
	500	1.301 ± 0.026	1.35 ± 0.066	92.0	1.32 ± 0.045	
Aqueous	100	0.235 ± 0.083	0.253 ± 0.073	22.4	0.373 ± 0.044	363.07
	200	0.344 ± 0.053	0.502 ± 0.022	32.3	0.618 ± 0.029	
	300	0.447 ± 0.023	0.772 ± 0.051	43.0	0.735 ± 0.059	
	400	0.788 ± 0.095	1.02 ± 0.091	56.0	0.948 ± 0.028	
	500	0.972 ± 0.056	1.20 ± 0.027	67.0	1.21 ± 0.055	
Vitamin C (Standard)	100			92.3	0.201 ± 0.028	57.93
	200			93.8	0.495 ± 0.035	
	300			95.1	0.697 ± 0.008	
	400			95.8	0.992 ± 0.072	
	500			96.7	1.201 ± 0.030	

Table 2. Antibacterial activity of leaf extracts of *Pistacia lentiscus* and standard antibiotics against tested bacterial isolates.

Solvent/ Antibiotic	Concentration (%)	Diameter of inhibition zone in mm			
		Gram positive bacteria		Gram negative bacteria	
		S. aureus	*S. haemolyticus*	*P. aeruginosa*	*P. mirabilis*
Methanol	25	26.00 ± 1.00 (S)	21.33 ± 1.15 (S)	15.00 ± 1.00 (I)	12.66 ± 0.57 (R)
	50	28.00 ± 1.73 (S)	24.66 ± 0.57(S)	15.33 ± 0.57 (I)	14.66 ± 0.57 (R)
	75	29.33 ± 1.15 (S)	27.00 ± 0.00 (S)	17.33 ± 0.57 (I)	16.00 ± 0.00 (I)
	100	33.33 ± 1.52 (S)	27.66 ± 1.52 (S)	18.66 ± 0.57 (I)	17.00 ± 1.00 (I)
Aqueous	25	13.66 ± 0.57 (I)	16.66 ± 0.57 (I)	0.00 ± 0.00 (R)	12.00 ± 1.00 (R)
	50	15.66 ± 0.57 (I)	18.00 ± 1.00 (I)	10.33 ± 0.57 (R)	13.66 ± 0.57 (R)
	75	19.33 ± 0.57 (I)	19.33 ± 0.57 (I)	11.00 ± 1.00 (R)	15.33 ± 0.57 (I)
	100	21.33 ± 0.57 (S)	23.00 ± 1.00 (S)	15.00 ± 1.00 (I)	18.33 ± 1.52 (I)
Levofloxacin (5 µg)		34.33 ± 0.57 (S)	33.00 ± 1.00 (S)	0.00 ± 0.00 (R)	36.33 ± 0.57 (S)
Ampicillin (10 µg)		15.33 ± 0.57 (R)	23.00 ± 0.00 (R)	0.00 ± 0.00 (R)	0.00 ± 0.00 (R)

Values are expressed as mean values ± SD (N = 3). Letters in parentheses represent the clinical breakpoints (susceptible (S), intermediate (I), and resistant (R)) as recommended by CLSI guideline protocols. Bacterial species: *S. aureus*; *Staphylococcus aureus*, *S. haemolyticus*; *Staphylococcus haemolyticus*, *P. aeruginosa*; *Pseudomonas aeruginosa* and *P. mirabilis*; *Proteus mirabilis*.

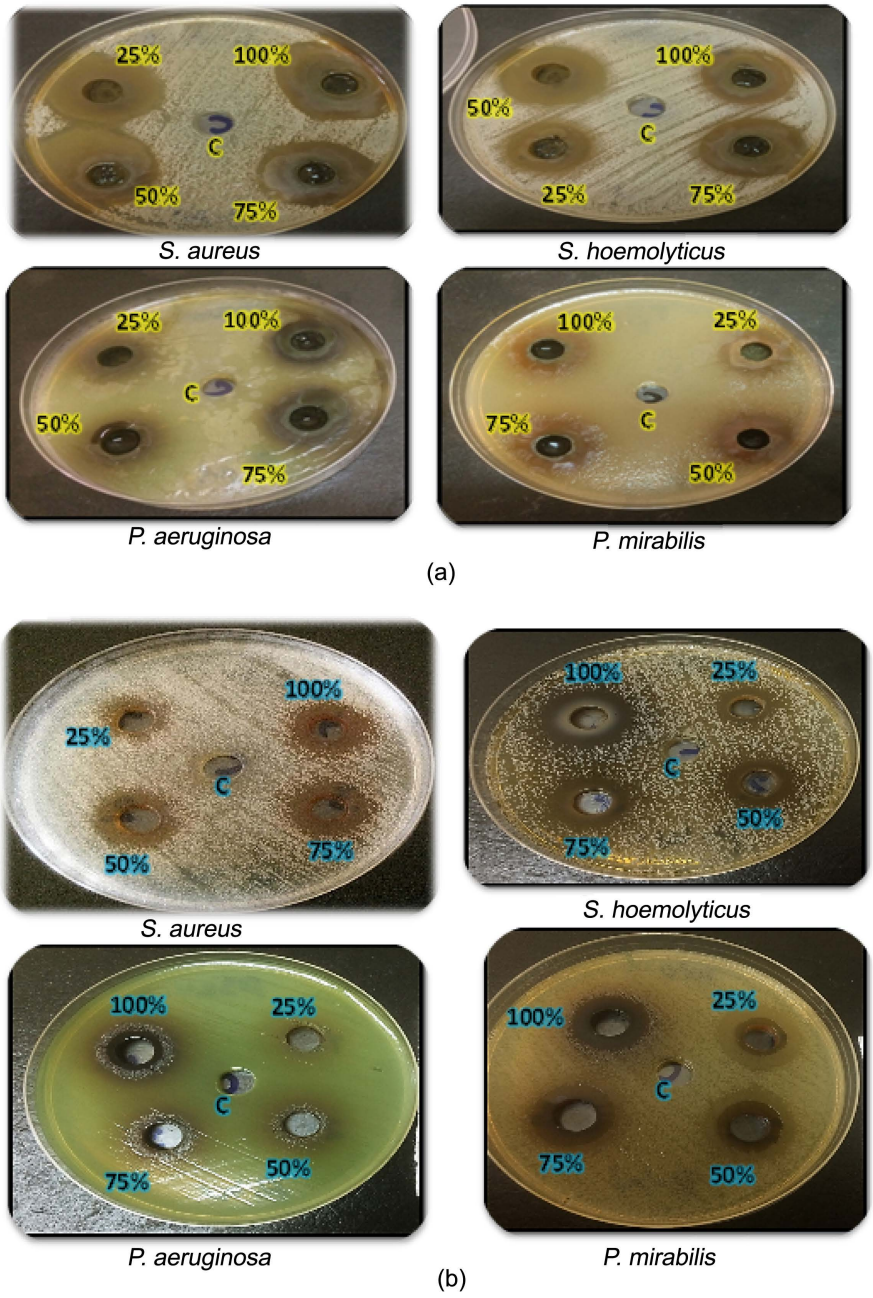


Figure 1. Antibacterial activity of *P. lentiscus* methanol (a) and aqueous (b) leaf extracts against *S. aureus*, *S. haemolyticus*, *P. aeruginosa*, and *P. mirabilis*.

methanol leaf extract showed a significant inhibitory effect on the growth of all tested bacterial isolates, presenting the higher inhibition zone than that of aqueous extracts at all concentrations studied (Table 2). The maximum zone of inhibition was observed in the methanol extract at the concentration of 100% with 33.33 mm and 27.66 mm against *S. aurous* and *S. haemolyticus*, respectively. Against the Gram positive bacteria both extracts were found effective at higher concentration. In addition, the results of this study indicated that the antibacterial inhibition activities were nearly similar for *P. aeruginosa*

and *P. mirabilis* and detected lowest inhibition zone of diameters, especially aqueous extracts at all the concentrations used. The results of the aqueous leaf extract also exhibited that there was no inhibitory activity against *P. aeruginosa* at the lowest concentration of 25. Our findings further indicated that Gram negative bacteria were less susceptible to both extracts compared to Gram positive bacteria.

Regarding antibacterial susceptibility testing results**,** all the Gram positive isolates showed highest susceptibility to standard antibiotic levofloxacin (100%), but highly resistant (100%) to ampicillin. On the other hand, all Gram negative isolates tested in this study recorded resistance against all standard antibiotics used, except *P. mirabilis* was levofloxacin susceptible (Table 2).

3.3. Reducing Power Capacity (RPC)

In this assay, the reducing capacity of both extracts and vitamin C were concentration dependent (Table 1). Increasing absorbance at 700 nm indicates an increase in reducing power activity. The results presented in our study revealed that both extracts showed good reducing power ability compared to the standard, vitamin C. The reducing power activity at lower (100 µg/ml) and higher (500 µg/ml) concentrations were (0.398; 1.32), (0.373; 1.21), and (0.201; 1.201) for methanol, aqueous, and vitamin C, respectively. Methanol leaf extract displayed a better reducing power capacity than that of aqueous extract and standard (Figure 2). There was no statistically significant difference between both leaf extracts and the reference vitamin C ($P > 0.05$).

3.4. DPPH˙ Radical Scavenging Activity (RSA)

The inhibition percentage of DPPH˙ radical scavenging activity is shown in Table 1. The percentage inhibition DPPH scavenging capacities of all parameters were increased in a concentration dependent manner. Furthermore, the radical scavenging activity of methanol and aqueous extracts were lower than the antioxidant capacity of the standard vitamin C at all concentration studied. At a final concentration of 500 µg/ml, the standard vitamin C displayed the highest

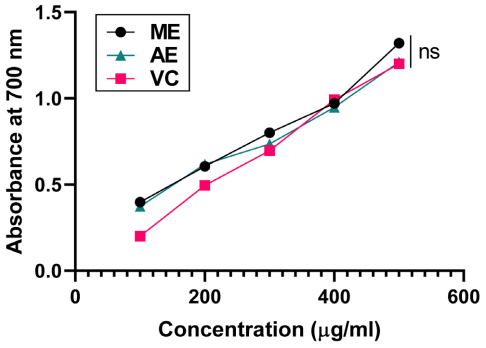


Figure 2. Reducing power of *P. lentiscus* extracts at different concentations. ME; methanolic extract, AE; aqueous extract, VC; vitmain C (positive standard), ns; no significant difference between both leaf extracts and the reference vitamin C ($P > 0.05$).

scavenging effect against DPPH with 96.7%, followed by methanol and aqueous leaves extracts (92% and 67%), respectively (Table 1 and Figure 3). There was statistically significant difference between both leaf extracts and the standard antioxidant vitamin C ($P < 0.01$).

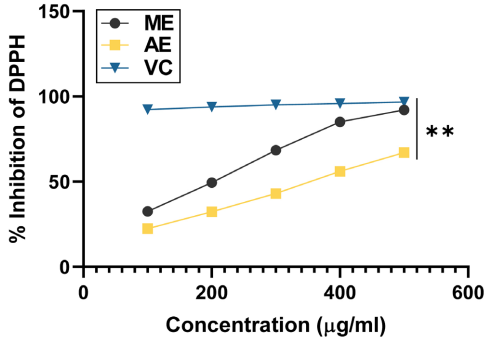
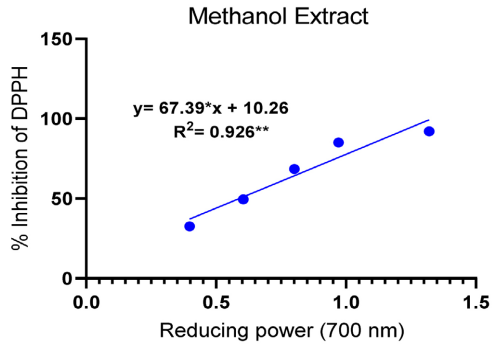


Figure 3. Antioxidant activity of ME and AE from leaves of *P. lentiscus* by DPPH free radical scavenging activity (% inhibition). ME; methanolic extract, AE; aqueous extract, VC; vitmain C (positive standard). **significant difference between both leaf extracts and the reference vitamin C ($P < 0.01$) by analysis of variance (one-way ANOVA) followed by Tukey' test (n = 3).

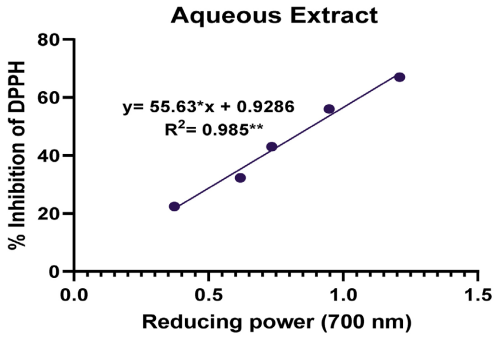
The antioxidant capacity of the extract was further estimated using the IC_{50} value, which determines the concentration of antioxidant substrate that inhibits DPPH activity by 50%. A lower IC_{50} value indicates greater ability to scavenge DPPH radicals. As shown in Table 1, vitamin C demonstrated significantly higher radical scavenging activity with the lowest IC_{50} value of 57.93 µg/ml when compared to methanol and aqueous extracts, which demonstrated inhibitory effects with IC_{50} values of 189.62 µg/ml and 363.07 µg/ml, respectively.

3.5. Correlation between Antioxidant Activities with Total Phenolic and Flavonoid Contents of *P. lentiscus* Leaf Extracts

Pearson's correlation coefficient (r) and coefficient of determination (R^2) were calculated to assess the relationship between total phenolic content (TPC) and total flavonoid content (TFC) and both reducing power capacity (RPC) and DPPH free radical scavenging activity (RSA). The antioxidant properties of plant extracts could be attributed to TPC and TFC. As shown in Table 3, the current study found a strong positive correlation ($r > 0.97$, $R^2 > 0.95$, $P < 0.01$) between total phenolic and flavonoid contents of *P. lentiscus* leaf extracts and both antioxidant activities tested (RSA and RPC). Furthermore, DPPH radical scavenging activity correlated strongly with reducing capacity (methanol extract, $R^2 = 0.926$, $P < 0.01$; aqueous extract, $R^2 = 0.985$, $P < 0.01$; vitamin C, $R^2 = 0.976$, $P < 0.01$) (Figures 4(a)-(c)). This relationship is consistent with the Pearson's correlation between TPC, TFC, and both antioxidant activities (Table 3), as evidenced by the high correlation coefficient of determination ($R^2 > 0.92$).



(a)



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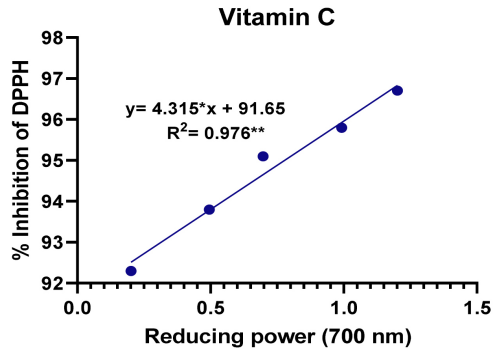

(c)

Figure 4. Linear relationship between reducing power capacity and DPPH free radical scavenging activity (% inhibition) in both leaf extracts of *P. lentiscus* compared with the standard antioxidant vitamin C. **Correlation is significant at $P < 0.01$ (two-tailed).

Table 3. Pearson's correlation coefficient (r) and coefficient of determination (R^2) between antioxidant assays (RSA and RPC) and total phenolic content (TPC) and Total flavonoid content of methanol and aqueous leaf extracts of *P. lentiscus*.

Antioxidant assays	Methanol extract		Aqueous extract	
	TPC	TFC	TPC	TFC
RSA	0.993*** (0.987)	0.979** (0.960)	0.995*** (0.991)	0.984** (0.968)
RPC	0.976** (0.952)	0.992*** (0.984)	0.984** (0.970)	0.977** (0.955)

Values in parentheses represent the R^2 values. **Correlation is significant at $P < 0.01$ (two-tailed); ***Correlation is significant at $P < 0.001$ (two-tailed). RSA, radical scavenging activity (*% inhibition of DPPH*); RPC, Reducing power capacity.

4. Discussion

Phytochemical compounds, primarily polyphenols such as flavonoids and phenolic acids, are abundant in plant products and have been shown to have significant antibacterial, antioxidant, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and vasodilatory activity. Many of these biological characteristics are primarily responsible for their antioxidant and free radical scavenging abilities [47] [48]. Methanol is a common solvent for extracting antimicrobial medicines, and it may contain a variety of chemical compounds with biological activities [49] [50].

According to our findings, the phytochemical analysis of both test extracts demonstrated the presence of considerable polyphenolic components, mainly phenolics and flavonoids. Furthermore, the chemical composition of *P. lentiscus* leaf extracts varied substantially depending on the test extracts and concentration. The total phenolics and flavonoids in the methanolic leaf extract were higher than in the aqueous extracts. This is consistent with prior research [29] [51] [52] [53] [54] that revealed methanol to be a good solvent for extracting bioactive chemicals from plants, such as phenolic and flavonoid components.

At all concentrations examined, the antibacterial effect of the methanol leaf extract of *P. lentiscus* was shown to be greater than that of the aqueous extract against all tested microbes. The diameters of the inhibition zones were utilized to track the susceptibility of the test microorganisms to plant extracts. *P. lentiscus* growth inhibition differed from one microbe to another, as well as from one plant extract to another. The zones of inhibition for methanol extract range from 14.6 to 33.3 mm and 10.3 to 22.6 mm for aqueous extract, depending on concentrations, however the aqueous extract had no significant inhibitory effect against *P. aeruginosa* isolate at the lowest concentration (25%). Despite having lower potency than the methanol extract, the water extract of *P. lentiscus* showed antibacterial effect.

Furthermore, Jayalakshmi *et al.* [53] examined the antibacterial efficacy of various solvent extracts of the different plants against the human pathogenic bacteria and concluded that methanol extract of almost all the screened plants showed activity against all microorganisms tested. Similarly, a further study, reported by Parekh *et al.* [55] revealed that methanol extracts are more active than aqueous extracts in their antibacterial effect. Additionally, other studies showed the efficacy of methanolic [56] [57] and the aqueous extracts [58] of the leaves of *P. lentiscus* against several pathogenic microbes due to the presence of the polyphenols compounds. In our previous in vitro study, we reported a significant antibacterial capacity against *S. aureus* and *P. aeruginosa* using acetone leaf extract for *P. lentiscus* [59], while another study indicated no antibacterial activity were observed from the acetone leaf extracts of *P. lentiscus* towards *K. pneumoneae* and *E. coli* [30].

Furthermore, Muzafar *et al.* [60] reported that, whereas certain plants' aqueous extracts had limited zones of inhibition, other plants displayed a substantial inhibitory effect against all of the target pathogens under the same extracts. In the

current investigation, Gram positive bacteria were shown to be substantially more vulnerable to the studied extracts and to have a greater inhibitory zone than Gram negative bacteria. High susceptibility of Gram positive bacteria, particularly *S. aureus*, to various plant extracts has also been reported in the literature [53] [61] [62].

In comparison to these findings, Farjana *et al.* [63] illustrated that at a concentration of 0.2 mg/ml, the methanolic extract of Azadirachta indica showed a lower inhibition zone (10mm) against *S. aureus*. The amount of concentration, extracting solvent, strain employed, and plant genotype may all have a role in these disparate findings. Gram negative bacteria's cell walls are less vulnerable to numerous phytoconstituents than Gram positive bacteria's due to the outer membrane layer, which serves as a protective barrier against harmful substances, including antibiotics.

Although many studies have shown that levofloxacin is effective in treating infections caused by *P. aeruginosa* [46] [64] [65] [66] [67], the *in vitro* activity of levofloxacin against isolate of *P. aeruginosa* did not show any inhibitory effect in our experiments. Atef *et al.* [68] have reported that *P. mirabilis* was susceptible to several antibiotics, including levofloxacin, which is consistent with the findings of this investigation. In the same experiment, one isolate (7.69%) of 13 *P. aeruginosa* strains proved resistant to levofloxacin. Uncontrolled usage in hospitals and communities may have contributed to the development and spread of antibiotic resistance.

Interestingly, the methanol leaf extract of *P. lentiscus* exhibited a broad-spectrum and potent antibacterial effect against ABR *P. aeruginosa* and *P. mirabilis* than that of antibiotics used. These Gram-negative bacteria, especially the *P. aeruginosa* isolate, were shown to be completely resistant to all antibiotics tested. The considerable effectiveness of *P. lentiscus* extract against these AB-R pathogenic bacterial isolates could provide an essential new treatment option for bacterial illnesses. Generally, the antibacterial effect of plant extracts, particularly methanol, can be attributed to phenolic and flavonoid chemicals, which have antagonistic effects on the microorganisms examined.

A DPPH (diphenylpicrylhydrazyl) and reducing antioxidant power methods were used *P. lentiscus* plant extracts. Has been recognized as a valid method for assessing plant extract antioxidant capability [69]. Free radicals can cause damage in vivo in a biological system and are thus thought to be a cause of a variety of diseases. As a result, in order to protect living systems, antioxidant molecules must [70]. In the current investigation, the extracts and the reference vitamin C, were examined for their ability to scavenge free radicals at varied concentrations. The percentage of inhibition, was measured property of the plant extract and standard which is able to scavenge or inhibit free radical. Our findings demonstrated that the inhibition percentage of DPPH scavenging capability of the leaf extracts and standard exhibited a considerable concentration dependent inhibition of DPPH activity.

Our findings also showed that at a concentration of 500 g/ml, the standard of

vitamin C and methanol extracts of *P. lentiscus* had strong radical-scavenging activity, with antioxidant percentages of 96.7% and 92% for vitamin C and methanol extract, respectively. Vitamin C, followed by methanol extract, had excellent antioxidant capacity (% DPPH radical inhibition) at all concentrations investigated of *P. lentiscus*, whereas aqueous extract had weak to moderate inhibitory effects. These results may elucidate that the extracts contain chemicals that appear to function as a better electron and hydrogen donating capabilities of these samples, and hence could be the source of their potent antioxidant action.

The reducing power assay, which is a key feature of antioxidant activity, is another assay that is connected with electron/hydrogen donating ability. The reducing power of antioxidants was determined using the potassium ferricyanide (Fe^{3+}) reduction method in the presence of methanolic and aqueous extracts of *P. lentiscus* leaves and a standard of vitamin C to examine the reductive ability of antioxidants by converting ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). An increase in reductive ability is indicated by an increase in absorbance at 700 nm. In accordance with the results presented for the percentage of DPPH radical inhibition, reducing power assay exhibited also antioxidant activity increased in a concentration dependent manner (**Table 1**). According to our findings, the reducing power capacity of both extracts, in particular, methanolic extract, showed higher reducing power than the synthetic vitamin C, indicating that the extracts may have a stronger antioxidant activity.

These findings corroborated prior findings that the methanolic leaf extracts of *P. lentiscus* had the highest antioxidant activity and reducing power utilizing DPPH radical scavenge and reducing power, which is characterized by the presence of phenolic and flavonoid constituents [29] [51] [71]. Due to their unique nature as important bioactive constituents of medicinal plants, phenolic compounds are recognized to be potent chain breaking and free radical terminator antioxidants [72].

The IC_{50} value is commonly used to assess the antioxidant activity of test samples. It is defined as the amount of substrate necessary to inhibit 50% of DPPH activity. As a result, the lower the IC_{50} value, the greater the antioxidant activity. According to Marjoni and Zulfisa [73], the potency antioxidant capability can be characterized as highly active (50 g/ml), active (50 - 100 μg/ml), moderate (101 - 250 μg/ml), weak (250 - 500 μg/ml), and inactive (>500 μg/ml) based on the IC_{50} value. The reference vitamin C had the highest antioxidant ability, with an IC_{50} value of 57.93 μg/ml, followed by methanol extract, which had an IC_{50} value of 189.62 μg/ml. The aqueous extract had the lowest radical scavenging activity with an IC_{50} value of 363.07 μg/ml.

The low activity of the aqueous extract could be attributed to the polyphenol oxidase enzyme, which hydrolyzes polyphenols in aqueous extracts, whereas in methanol is not active [74]. The high polarity of the solvent has been demonstrated to have a negative impact on plant material activity [75], decrease in total phenolic and flavonoid content of the extracts, and hence a decline in antioxidant activity [76] [77]. The findings of the present investigation indicated that

although aqueous extract showed a weak potent DPPH radical scavenging with IC50 value of 363.07 μg/ml, its activity was reported, which is consistent with previous studies [73] [78], showing that an IC50 value of 200 - 1000 μg/ml was declared less active but still has potential as an antioxidant.

Accordingly, polyphenols, mainly phenolic and flavonoid constituents can contribute directly to the potential antioxidant properties. These polyphenol compounds mainly can act as antioxidants by various approaches such as reducing agents, hydrogen donators, and free radicals scavengers, thereby reducing the redox potential of the medium by chelating pro-oxidant metal ions and inhibiting some enzymes, which may also restrict the growth of microbial pathogens [79] [80]. As indicated in **Table 3**, the current study found a strong association between total phenolic and flavonoid contents and RSA (% DPPH inhibition) and RPC (reducing power capacity). Several studies have found a direct association between total phenolic and flavonoid content and the antioxidant capacity of plant extracts [81] [82], whilst others have found no correlation between total phenolic [83] or flavonoid [84] content and their in vitro antioxidant activities.

Furthermore, our results reported that there was a significant linear correlation ($r > 0.97$, $R^2 > 0.93$, $P < 0.01$) between total phenolic and flavonoid contents with their antioxidant activities of both extracts and standard. In addition, a linear relationship was observed between DPPH free radical scavenging activity (% inhibition) and reducing power capacity in both extracts and the reference vitamin C (**Figures 4(a)-(c)**). Therefore, the present study has suggested that the phenolic and flavonoid constitutes either individual or synergistic interactions contribute significantly to the antioxidant and antibacterial capacities of the *P. lentiscus* leaf extracts. In comparison to the aqueous extract, the methanolic extract revealed a significant source of antibacterial and antioxidant activity.

A limitation of our study is that we were unable to measure the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione reductase, which provide a greater knowledge about the mechanisms involved in the prevention of oxidative stress by some plant extracts possessing antioxidant properties.

5. Conclusion

The methanolic extract exhibited strong antibacterial and antioxidant properties than that of the aqueous extract. This could be due to the methanolic extract having the highest concentration of total phenolic and flavonoid compounds. As a result, more active component isolation and characterization, as well as *in vivo* research, are needed to determine the mechanism of antibacterial and antioxidant effects.

Conflicts of Interest

The authors declare no conflicts of interest.

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