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Evaluation the *in vitro* antibacterial activity of acetone leaf extracts from *Pistacia lentiscus* against multi-drug resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Abstract
Since ancient decades, *Pistacia lentiscus* is known by its immense medicinal qualities. The leaves of the *Pistacia lentiscus* plant possess a multitude of biological activities, such as antioxidant, antimicrobial, antitumor, anti-inflammatory, anticancer, antiradical, etc. The present study was conducted to evaluate the phytochemical proprieties, mainly presented by the presence of pyrogallol and qurecetin, which considered as the most important bioactive compounds with antioxidative and antimicrobial properties and then assess the antibacterial activity of acetone leaf extract of *Pistacia lentiscus* on two pathogenic bacteria namely *Staphylococcus aureus* and *Pseudomonas aeruginosa* using agar well diffusion method. Our results indicated that the total contents of pyrogallol and qurecetin from the acetone extract of *Pistacia lentiscus* leaves were gradually increased with increasing concentrations, which obviously appeared as a positive and highly significant linear correlation between absorbance and concentration (pyrogallol / qurecetin: Coefficients of determination (R^2) = 0.9908 / 0.9978, respectively). The results of the present work revealed that at all concentrations, the acetone leaf extract of *P. lentiscus* recorded different degrees of antibacterial activity against both bacteria. Therefore, we can conclude that the antibacterial activity of the plant under investigation may be as a result of the bioactive compounds present.

Keywords: *Pistacia lentiscus*, multidrug resistant, quercetin, pyrogallol, antibacterial activity

1. Introduction
In recent years, the bacterial resistance to antimicrobial is globally becoming a great challenge due to the capability of bacteria to resist many of the presently available antibiotics. Multidrug-resistant (MDR) *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) are considered the most important pathogens responsible for nosocomial infections, which result in high mortality and morbidity rates [1-5]. As a consequence, the search for complementary and alternate therapeutics has become a vital for discovery of MDR in bacteria due to the clinical failure of several usual antibacterial in controlling an affronting MDR pathogen; however, natural drugs and herbal therapies can suggest some alternative techniques [6-9]. MDR is a major threat to human health worldwide, and also to crops and animals. MDR is a rising challenge in medicine [10].
According to World Health Organization, medicinal plants would be the best source to obtain a wide variety of bioactive compounds [11]. *Pistacia lentiscus* L. (*P. lentiscus*), commonly known as Mastic, is an evergreen shrub from Anacardiaceae family, widely distributed throughout the Mediterranean area [12-15]. Furthermore, its mastic has been utilized in folk medicine since the time of the ancient Greeks [16]. Moreover, the aerial part of this plant has traditionally been utilized as a stimulant, for its diuretic activities, and to treat hypertension, coughs, sore throats, eczema, stomach aches, kidney stones and jaundice [17-20] because of strong antioxidant [21], anti-inflammatory and antimicrobial effects [22, 14, 23].
Therefore, nowadays, the development of bioactive compounds for antibacterial treatments that are innovative and add significant clinical value is critical need to prevent the spread of diseases and enhance their treatment. To our knowledge from the literatures, little research has been conducted on determining the antibacterial activity of *P. lentiscus* plant in Libya. In addition, several phytochemical studies have been reported the use of plant extracts as effective antimicrobial drugs, using various extraction solvents, however, the evaluation of acetone efficiency is scarce. Consequently, the current research was conducted to highlight the

chemical composition of *P. lentiscus* leaves by the presence of pyrogallol and quercetin compounds and then to evaluate the effect of acetone extract *P. lentiscus* leaves toward pathogenic multidrug-resistant bacteria.

2. Materials and Methods

2.1 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. Botanical identification was made by the member of herbarium of Botany department (Faculty of Science, University of Benghazi).

2.2 Preparation of the extracts

The extract was obtained by macerating 30 g of the dried leaves from *P. lentiscus* separately in acetone (300 mL) for 48 h. The resultant extract was filtered under vacuum and the solvent was evaporated to dryness using a rotary evaporator. The dry extract was stored at 4 °C until further use.

2.3 Phytochemical Analysis

2.3.1 Determination of Total Flavonoid Content (TFC):

Total flavonoid content was determined using the aluminum chloride colorimetric method of [24]. A volume of 2 ml of different concentration "100, 200, 300, 400, 500 µg/ml "of the extract was mixed with 0.1ml of a 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was obtained by preparing different quercetin solutions in acetone at concentrations "100 - 500 µg/ml". The results were expressed as µg of quercetin equivalents/ml of *P. lentiscus* extract. Estimation of flavonoid compound (quercetin) was carried out in triplicate.

2.3.2 Determination of Total Phenolic Content (TPC)

Total concentration of phenolic compound in the acetone extract obtained from *P. lentiscus* leaves was estimated using the colorimetric method based on Folin-Ciocalteu reagent [25]. Briefly, 0.05 ml of different concentrations "100, 200, 300, 400, and 500 µg/ml" were mixed separately with 0.05 ml of Folin-Ciocalteu reagent. Then 0.5 ml of 15% sodium carbonate solution was added to the mixture and then the adjusted to 1 ml with 0.4 ml of distilled water. The reaction was allowed to stand for 10 min, after which the absorbance were recorded at 725 nm by UV-visible spectrophotometer. The results were expressed as µg of pyrogallol equivalents/ml of *P. lentiscus* extract. The experiments performed in triplicate were expressed as mean values ± standard deviations (SD).

2.4 Microorganisms

The bacteria used in the *in vitro* test were *P. aeruginosa* and *S. aureus* and were obtained from the laboratory of microbiology in Benghazi Children's Hospital, which know as multi-drag resistant bacteria. The microorganisms were isolated and identified in El-Jala Teaching Hospital using standard methods, and identification confirmed via utilizing phoenix.

2.5 Assessment of antibacterial activity of the extraction

In the present study, diluted of leaves extracts from the *P. lentiscus* were used. The diluted extracts leaves were prepared by using Dimethyl Sulfoxide (DMSO) to obtain 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v) concentrations. DMSO was considered as negative control. The antibacterial activity of the extract leaves were tested by agar well diffusion method [26]. Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100 µl bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation, 9 mm diameter holes were punched into the surface of the agar using a sterile cork borer. Different concentrations (25, 50, 75 and 100%) were added to the wells. Plates were incubated at 37 °C for 24 h. The diameter of the zone of inhibition was measured via using a ruler in mm.

2.6 Statistical analysis

The statistical analysis was performed by one-way ANOVA using SAS® University Edition software with PROC MIXED procedure (SAS®, Institute Inc., Cary, NC, USA). Differences among treatments were compared using Tukey's HSD test**.** Results were considered statistically significant at $P < 0.05$. The results were expressed as mean ± SD of three independent replicates.

3. Results

3.1. Phytochemical Analysis

The results of phytochemical screening revealed that the natural phenolic compound pyrogallol and flavonoid compound qurecetin were chemically observed. Our findings of the present study indicated that the contents of pyrogallol and qurecetin in the acetone extract were gradually increased with increasing concentration, which obviously appeared as a linear and positive relationship between absorbance and concentration (pyrogallol / qurecetin: Coefficients of determination (R^2) = 0.9908 / 0.9978, respectively) (Fig. 1 a and b).

3.2 Antibacterial activity

The *in vitro* antibacterial activity of the leaf acetone extract of *P. lentiscus* was evaluated against two multidrug resistant gram-positive and gram-negative bacteria using hole diffusion method. The obtained results for the screening of antibacterial activity measure as a zone of inhibition of acetone extract of *P. lentiscus* plant have shown in Table 1 and represented by the Figure 2. Moreover, the plant substances were tested in various concentrations. At all concentrations, the acetone leaf extract of *P. lentiscus* showed different degrees of antibacterial activity against tested microorganisms as evidenced by the zone of inhibition (Table 1 and Fig. 2).

Analysis of variance (ANOVA) showed that the highest antibacterial activity with maximum zone of inhibition (27.66 mm) was recorded against *S. aureus* as compared to the respective lowest concentration (15 mm). Extract also showed good inhibition zone (17.33 mm) towards *P. aeruginosa* for the highest leaf concentration used in this study, however, negative control showed no observable inhibitory effect against any of the pathogenic bacteria tested (Table 1 and Fig. 2). Additionally, there was a highly significant bacteria × concentration interaction ($P = 0.0001$) in the mixed-model analysis of variance (ANOVA). It was observed in this study that antibacterial activity of the plant extract increased with the increase in the concentration used (Table 1 and Fig. 3). In general, the present work clearly indicated that 100% acetone extract showed the highest antibacterial activity against tested bacterial strains.

4. Discussion

In recent years, the development of microbial resistance to available antibacterial has increased and considered to be the major threat to public health. As a consequence, search for alternative strategy with more effective and secure to combat critical bacterial infections is urgently required. Medicinal plants, as a rich source of bioactive components could be a good candidate for this task. *P. lentiscus* leaves contain various kinds of bioactive metabolites. Among them, the most abundant compounds in *P. lentiscus* leaves are reportedly flavonoids, which have displayed a potent antioxidant activity [27] as well as anti-inflammatory and anticancer effects [28, 29]. Liu *et al*. [30] have been reported that quercetin as a flavonoid compound is a well-known antioxidant which has many beneficial health effects such as antiviral, anti-inflammatory, antibacterial, and muscle-relaxing properties.

Similarity, Cai *et al*. [31] have found that the quercetin, kaempferol, myricetin, and their glycosides were the predominant flavonols, occurring in several medicinal herbs associated with anticancer. On the other hand, pyrogallol as a phenolic component has also reported to have antibacterial [32] and display high superoxide radical scavenging [33] activities. Pervious phytochemical studies on the leaf extracts of *P. lentiscus* reported that the chloroform, ethyl acetate and aqueous leaf extracts exhibited the bioactive metabolites such as flavonoid (quercetin) and phenolic compounds [13]. Elmhdwi *et al*. [34] have illustrated that the highest extraction rate of phenolic and flavonoid compounds for *Juniperus phoenicea* was obtained by acetone as compared to other solvents. Furthermore, Benhammou *et al*. [35] pointed out in their study that the use of ethanol solvent allows the extraction of phenolic compounds from the leaves of different *Pistacia* species, including *P. lentiscus*.

The bioactive compounds are extensively found at different parts and levels in many medicinal plants, and therefore, the present study was carried out to explore the phytochemical composition of the leaf extract of *P. lentiscus* and also to assess the antibacterial activity against two species of Gram-positive and negative bacteria. In addition, several phytochemical studies have been reported the use of plant extracts as effective antimicrobial drugs, using different extraction solvents, but the evaluation of acetone efficiency still continues being scarce. Furthermore, Eloff [36] has reported that the extraction with acetone is considered the best choice because it can extract metabolites of a wide array of polarities, it is nontoxic to bioassay systems and easy to eliminate from extracts. Thus, the current research had two objectives: the first, to evaluate the presence of phenolic and flavonoid compounds, especially pyrogallol and quercetin from acetone leaf extract of *Pistacia lentiscus* and second, to determine whether these compounds have an antibacterial capacity on two pathogenic bacteria notorious for their resistant to available antibiotics.

Total phenolic and flavonoid contents of the acetone extract of *P. lentiscus* were estimated using the Folin–Ciocalteu colorimetric and aluminum chloride methods, respectively. The results of present study indicated that the phytochemical compounds from leaf extracts of *P. lentiscus* had highest content of phenolic and flavonoid compounds as expressed according to pyrogallol and qurecetin, respectively, which are known to exhibit antibacterial and antioxidant activities. This is in accordance with the results obtained from similar study by Saliha *et al*. [13] who reported that crud and ethyl acetate extracts of *P. lentiscus* showed highest amount of phenolic and flavonoid compounds as expressed by gallic acid and qurecetin, respectively.

As shown in Fig. 1a and b, the total pyrogallol and qurecetin contents increased with increasing extract concentration. In addition, our results revealed that there was a linear and positive relationship between absorbance and concentration, the correlation coefficients (R^2) were 0.9908 and 0.9978 with UV-visible spectrophotometer of 725 nm and 415 nm, for pyrogallol and qurecetin, respectively. Research in recent years reported that the ethyl acetate extract contains the highest content of flavonoids (mg Quercetin/g of dry ethyl acetate extract) and showed a strong absorption at 760 nm, whereas crude extract of *P. lentiscus* showed significantly higher amount of phenolics (mg Gallic acids equivalent/g dry crud extract) with maximum absorbance at 760 nm [13].

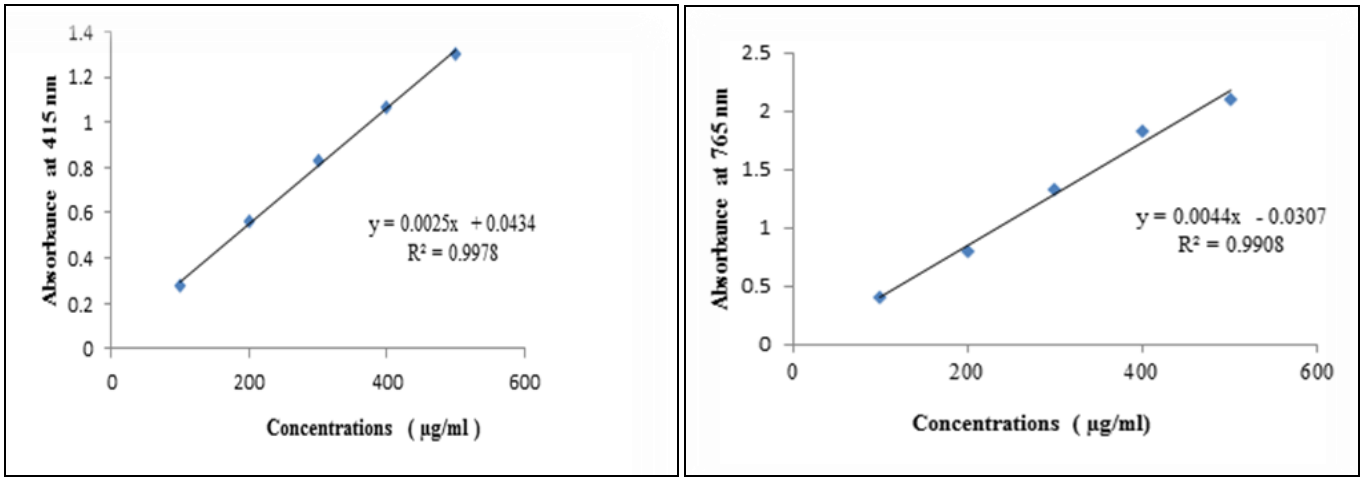
Moreover, the antibacterial activities of these phytochemical compounds were evaluated by testing their inhibitory ability on two bacterial species via using a hole diffusion assay. Like tested compounds, the antibacterial activity of the acetone extract was also concentration-dependent. The results indicate that the acetone extract of *P. lentiscus* leaves showed a highly significant ($P < 0.001$) inhibited towards tested bacteria. In addition, our findings showed that increase in concentration of the leaf extract increased the activity of the leaf plant extract on studied microorganisms (Table 1 and Fig. 3). These results were in concurrence with previous studies which illustrated that the bioactive compounds from plant extracts have been reported to have antibacterial in nature [37-39].

The antibacterial activity exhibited via *P. lentiscus* leaves against the tested bacterial strains might be due to the presence of bioactive metabolites such as quercetin and pyrogallol which are known to have antimicrobial effects [30, 32, 40-42]. To the best of our knowledge this study is therefore the first report on the acetone leaf extract of *P. lentiscus* in Libya. On the basis of the obtained results in the current study, it seemed that the potent antibacterial activity of acetone leaf extract of *P. lentiscus* was the result of a single or combined effect of these phytochemical constituents.

Table 1: Antibacterial activity of *P. lentiscus* leaf extract against test bacterial strains

Extract	Concentration (%)	Zone of inhibition (mm)	
		S. aureus	***P. aeruginosa***
Acetone	5	15.00±1.00	11.00±1.00
	25	19.33±0.57	15.33±0.57
	50	21.33±1.52	16.00±1.00
	75	22.33±1.52	16.66±0.57
	100	27.66±0.57	17.33±1.54
DMSO (control)		0.00±0.00	0.00+0.00

Values are expressed as mean ± SD (n=3) for each concentration. DMSO: Dimethyl Sulfoxide



a. b.

Fig 1: Linear correlations for total flavonoid content of quercetin (a) and for total phenolic content of pyrogallol (b) in acetone extract of *P. lentiscus* leaves at different concentrations

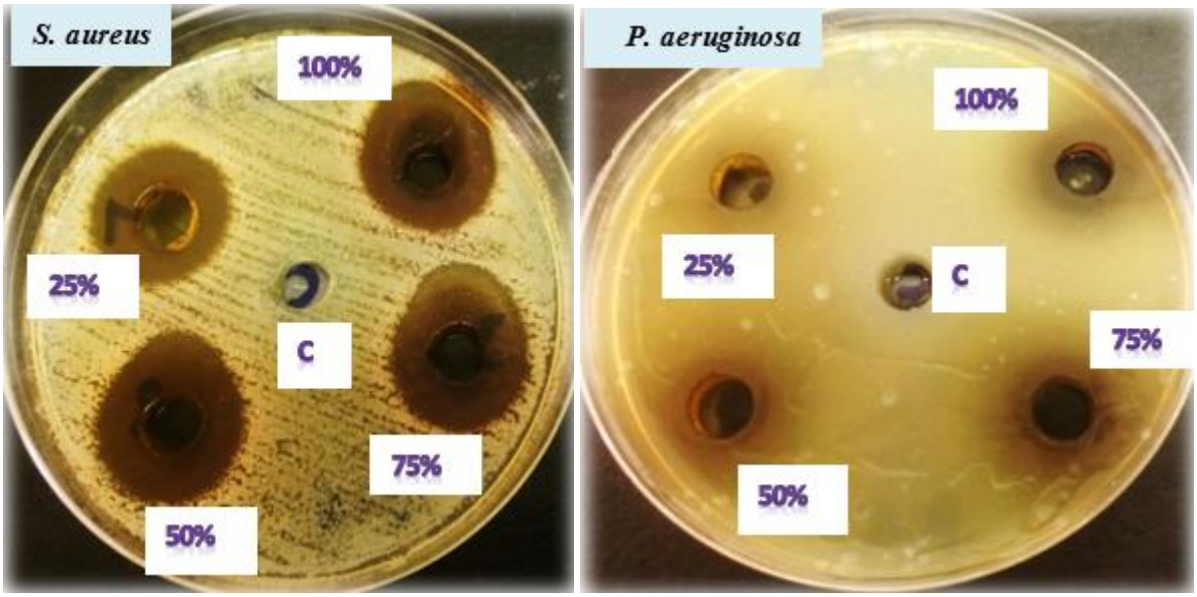


Fig 2: Zone of antibacterial inhibition of *P. lentiscus* leaves extract against *S. aureus* and *P. aeruginosa* isolates and the control (C- DMSO).

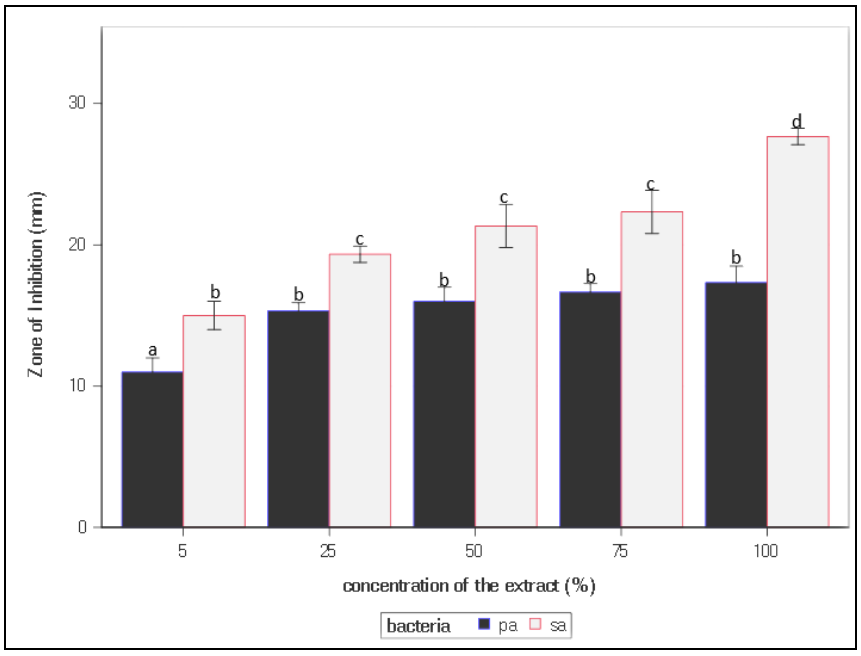


Fig 3: The inhibitory effect of different concentrations of acetone extract of *P. lentiscus* leaves on two pathogenic strains (*Staphylococcus aureus, Pseudomonas aeruginosa*). Data are presented as means ± SD of three independent replicates. Columns with different letters differ significantly using the Tukey's HSD test ($P < 0.05$).

5. Conclusion

Studies based on extraction of bioactive compounds from different part of the plant used for medicinal purposes are intensively increased in the last decades. Medicinal plants are massively used in traditional medicine for the treatment of infectious diseases, which appears to be very promising potential approach for identifying secure and effective antimicrobial drug candidates. In general, the study of antibacterial properties from plant materials appeared to depend on solvent used in plant extraction and its concentration. Our results indicated that the acetone extract of *P. lentiscus* can be utilized as a potential source of antibacterial compound and the presence of quercetin and pyrogallol might be possessing powerful natural antibacterial potential and play critical roles in preventing and treating infectious diseases. The results obtained from this research encourage investigations on Mediterranean plant species as sources of antibacterial agents.

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