Antibacterial effect of the extracts and essential oils prepared from *Pistacia lentiscus* L. mastic gum against pathogenic bacteria

Hassan F. Al-Mola

Department of Biology, College of Education, University of Mosul, Mosul, Iraq.
E-mail: hassssanbac@gmail.com.

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Abstract:

The water, ethanol, chloroform, acetone extracts and isolated essential oil of the mastic gum resins of *Pistacia lentiscus* L. were investigated for their antibacterial activity against seven different species of pathogenic microorganisms: *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Salmonella typhi* and *Pseudomonas aeruginosa* using disc diffusion and microdilution assay. All extracts showed antibacterial activity against most tested bacteria except, *P. mirabilis* and *E. coli* which were resistant to chloroform and acetone extracts. The ethanol extract exhibited the highest antibacterial activity against *S. aureus* whereas the water extracts exhibited some degree of activity and the chloroform and acetone extracts exhibited no or least activity. The isolated compound (essential oil) with a concentration of 50 mg/ml achieved good antibacterial activity against all tested bacteria (zone of inhibition range: 18-27 mm).Minimal inhibitory concentrations of The ethanol extract achieved best results against all tested bacteria and *S. aureus* was the most susceptible bacteria with a MIC value of 0.097 mg/ml, whereas the lowest susceptibility results were seen against *C. diphtheria* using the aqueous and acetone extracts. Finally the essential oil showed best results against *P. aeruginosa* and the least activity was calculated against *E. coli* and *S. typhi*.

Key words: Antibacterial ,Mastic gum, *Pistacia lentiscus* L.

Introduction:

More and more drugs are becoming useless as a result of increasing numbers of drug resistant pathogenic bacterial strains. The urge to find new active drugs, pure or modified, has become a critical task to overcome the limitations that older, still in use, drugs have faced. Drug companies and research facilities are screening different sources with different techniques to fill this need. For a successful screening process, optimized high-quality methods are needed. Natural products are still mostly an unexplored research area with a great potential for drug discovery. Major sources include marine organisms and microorganisms, among the fungi which are well-known as a source of antimicrobials. Also insects and animals, mostly venomous, are large but poorly characterized reservoirs [1,2,3]. Plants have been the largest natural source of new drugs, though only less than 5% of known plants have been chemically characterized. Especially extracts from plants can have significant value in antimicrobial research as they may inhibit bacterial growth by different mechanisms than conventional antibiotics. For example, plant extracts that contain different phenolics have shown good antimicrobial effects and are receiving growing interest [4,5,6,7].

Pistacia species have a wide range of uses in food industries [8,9]. The *Pistacia* resin is used in adherent production, in protecting luster for arts of glass, porcelain, bone, wood and metal. It is also used in alcoholic and non-alcoholic refreshments, in some cosmetic mixtures and perfumery; as an ingredient of filling material in dentistry and in toothpaste production [10]. Resin is also traditionally used as chewing gum, against lip-dryness, some stomach diseases and antiseptic for respiratory system [10,11]. *Pistacia* species are used in eczema treatment, paralysis, diarrheic, throat infections, renal stones, jaundice, asthma and stomach-ache, and as astringent, anti- inflammatory, antipyretic, antibacterial, antiviral, pectoral and stimulant [12,13,14,15]. Investigation on *Pistacia* species has also revealed that crude extracts, essential oils and some triterpenoid constituents exhibit anti-inflammatory, antifungal and antifeedant activities [16,17,18].

Mastic is a natural resinous exudate obtained from the stem and main leaves of *P. lentiscus* var. chia, a small evergreen tree that is cultivated almost exclusively on the Island .It is used as a food ingredient in the Mediterranean region, and has been used by local inhabitants as a traditional medicine for relief of upper abdominal discomfort, dyspepsia and peptic ulcer. Clinically, mastic has been effective in the treatment of benign gastric[19] and duodenal ulcers[20], giving symptomatic relief and endoscopically proven healing. Mastic has also shown cytoprotective and mild antisecretory properties on experimentally induced gastric and duodenal ulcers in rats[21]. The in vitro antibacterial activity of mastic against *H. pylori* could also contribute to its therapeutic effect in patients with peptic ulcers[22,23].

The leaves of *P. lentiscus* L. are used in eczema treatment, paralysis, diarrhea, throat infections, renal stones, jaundice, asthma, stomach- ache, as astringent, anti-inflammatory, antipyretic, pectoral and stimulant [24]. The fat extracted from *P. terebinthus* fruits is consumed in food and also used as raw material in soap production in some regions of Turkey [11]. Epilupeol and epilupeol acetate found in the resin of *Pistacia* species have antiviral activity against some virus in chicken embryo [25]. Bark of *P. lentiscus* has been widely used as a traditional folk

medicine against hypertension in some regions of Spain [26]. In addition, the resin of *P. lentiscus* has antioxidant and antimicrobial activities [27,16].

The objective of this study was to elucidate antibacterial activities of *P. lentiscus* mastic gum using different extracts, including isolated essential oil among various types of bacteria.

MATERIALS AND METHODS :

Mastic gum

Mastic gum the concrete resinous exudate from the stem of the tree *P. lentiscus* L. that is cultivated in Aegean and Mediterranean coasts of Turkey. Mastic gum samples were obtained from the grand bazaar of Istanbul. They have been collected from the Fethiye region, Turkey in June 2005.

Preparation of extracts:

Powdered samples (100g) were extracted with water, ethanol, chloroform and finally acetone using a soxhlet extractor. Each extraction was carried out for 8-10 h continuously. The solvents were then removed using a rotary vacuum evaporator at 40 °C to give concentrated extracts, which were frozen and freeze-dried until use.

Preparation of the essential oil :

The essential oil of the gums of *P. lentiscus* L. was prepared by steam distillation and the prepared oil was subjected to thin layer chromatography analysis as follows: Thin layer chromatography of the essential oil was chromatographed on silica gel (Whatmann Plates of TLC HP-KF, High Performance, Silica Gel, layer thickness 200 µ) using benzene-ethyl acetate (95:5) as developer. The spots were visualized by spraying with vanillin sulphuric acid reagent [28] and heating the plates at 120°C until the spots attain maximum color intensity.

Preparation of extract concentrations:

A volume of (0.25g) from each extract (ethanol, aqueous, chloroform , acetone and essential oil) was dissolved in 5ml dimethylsulfoxide "DMSO" to produce a final concentration of 50 mg/ml which was used as a stock concentration in providing next dilutions (25, 12.5, 6.25 and 3.12 mg/ml), all extracts were sterilized by filtration through a 0.45 µm membrane filter[29].

The bacteria:

The following microorganisms were used in this study: *S. aureus*, *C. diphtheria*, *E. coli*, *P. mirabilis*, *K. pneumonia*, *S. typhi* and *P. aeruginosa*. All microorganisms were clinical isolates obtained from the bacterial collection of Department of Biology, College of Science, University of Mosul, Iraq. Microorganisms were further identified by current standard microbiological methods according to, [30] to insure.

Screening of antibacterial activity:

The mastic gum extracts and it's essential oil were screened for their antibacterial activity using the disc diffusion assay described by [31].100 µl of prepared culture was spread on surfaces of Mueller–Hinton agar (MHA). Sterile filter paper discs (Whatmann No.1: 6mm in diameter) were soaked with different concentrations of both extracts and compound (0.1 ml of extract/10 paper disc), then placed on the surface of the inoculated media plates slightly, antibiotic discs (Bioanalyse) 6 mm in diameter of (Gentamycin 10µg and Cefalexin 30µg)were used as positive controls. Spread plates were then kept at room temperature for 30 minute to allow diffusion and absorption of extracts prior to incubation at 37°C for 24 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

Microdilution assay:

The minimal inhibitory concentration (MIC) of the gum extracts and essential oil were determined based on a microdilution method in 96 multi-well microtiter plates, as previously described by [32] and modified by [33] Briefly, the dissolved materials were first diluted to the highest concentration to be tested (3.12 mg/ml), 50 µl of Nutrient broth was distributed from the 2nd to the 9th well, a volume of 100 µl from each of the extract and compound initially prepared was pipetted into the 1st test wells of each microtiter line, and then 50 µl of scalar dilution was transferred from the 2nd to the 9th well. To each well was added 10 µl of resazurin indicator solution (prepared by dissolving a 270-mg tablet in 40 ml of sterile distilled water). Finally, 10 µl of bacterial suspension was added to each well. The final concentration of the extracts adopted to evaluate antibacterial activity was included from 3.12 mg/ml to 0.006 mg/ml. Two columns in each plate were used as positive controls containing antibiotics (gentamycin and cefalexin in serial dilutions of 3.12-0.006 mg/ml). Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37 °C for 18-24 h. Color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of 3 values was calculated and that was the MIC for the test material.

RESULTS:

Results of antibacterial screening from gums of *P. lentiscus* L. extracts are presented in Table1. Inhibitory effects were between very high effects and no activity. *S. aureus* showed highest susceptibility against the aqueous extract using the extract concentration 50 mg/ml and a direct relation was seen between inhibitory effects and extract concentrations. Meanwhile, the weakest aqueous extract action was seen against *C.diphtheria* with an inhibition zone of 11 mm. Ethanol extract revealed promising results against most tested bacteria. Very clear inhibition zones were observed against *S. aureus, P. aeruginosa* and *S. typhi* (23, 22 and 20 mm respectively) followed by *C. diphtheria, E. coli, K. pneumonia* and finally *P. mirabilis* with inhibition zone 13 mm in diameter.

The acetone extract had moderate antibacterial activity against all tested bacteria except *E. coli* and *P. mirabilis*. Inhibition zones observed were between 7-18 mm in diameter and the highest effect was against *S. aureus* and *P. aeruginosa* using the extract concentration 50 mg/ml.

The aqueous extract demonstrated good inhibitory results against most tested bacteria and the highest growth inhibition (21 mm) was seen against *P. aeruginosa* using the highest extract concentration. Furthermore, *P. mirabilis* and *E. coli* were resisted all acetone and chloroform extract concentrations, meanwhile *K. pneumonia* resisted chloroform extracts.

The isolated essential oil with a concentration of 50 mg/ml achieved good antibacterial activity against all tested bacteria (Table 2). The best zone of inhibition was seen against *S. aureus* and *P. aeruginosa* (26 and 27 mm in diameter respectively). *E. coli* showed least susceptibility against the compound and was inhibited with a zone of inhibition (18 mm in diameter).

Table 1: Antibacterial activity of different extracts from *P. lentiscus*L. gum.

Microorganisms	Extract type	Zone of inhibition (mm)						
		Extract concentrations (mg/ml)					Control (µg/ml)	
		50	25	12.5	6.25	3.12	G	C
S. aureus	Aq E A C	18 25 16 12	16 23 14 10	13 19 12 7	11 17 9 –	9 15 7 –	28	23
C. diphtheria	Aq E A C	17 21 16 11	14 20 15 10	11 17 13 –	8 15 9 –	– 13 – –	23	28
E. coli	Aq E A C	12 18 – –	10 16 – –	8 13 – –	7 10 – –	– 8 – –	20	28
P. mirabilis	Aq E A C	10 13 – –	8 12 – –	– 10 – –	– 9 – –	– – – –	19	25
K. pneumonia	Aq E A C	15 17 13 –	13 15 11 –	9 12 9 –	– 10 – –	– 8 – –	22	30
S. typhi	Aq E A C	18 22 17 11	15 19 15 9	13 17 13 8	10 15 10 7	8 12 8 –	25	26
P. aeruginosa	Aq E A C	21 24 18 13	20 22 16 11	18 19 14 9	16 17 12 –	13 14 9 –	٢٤	21

Abbreviations: Aq: Aqueous, E: Ethanol, A: Acetone, C: Chloroform, G: Gentamycin (10 µg), C: Cefalexin (30 µg), –: No activity.

Table 2:Antibacterial activity of essential oil isolated from *P. lentiscus* L. gum.

Microorganisms	Zone of inhibition (mm) essential oil 50 mg/ml
S. aureus	26
C. diphtheria	25
E. coli	18
P. mirabilis	20
K. pneumonia	22
S. typhi	24
P. aeruginosa	27

Table 3 shows the minimal inhibitory concentrations (MIC) for the extracts and essential oil of *P. lentiscus*. All microorganisms tested were resistance against the chloroform extract. Moreover, *E. coli*, *P. mirabilis* and *K. pneumonia* resisted the aqueous and acetone extracts. The ethanol extract achieved best results against all tested bacteria and *S. aureus* was the most susceptible bacteria with a MIC value of 0.097 mg/ml, whereas the lowest susceptibility results were seen against *C. diphtheria* using the aqueous and acetone extracts. The essential oil showed best results against *P. aeruginosa* and the least activity was calculated against *E. coli* and *S. typhi*. In general the positive controls (Gentamycin and Cefalexin) were able to inhibit all tested bacteria. Cefalexin was more efficient than gentamycine against most tested bacteria.

Table 3: Minimal inhibitory concentrations of *P. lentiscus* L. gum extracts and essential oil.

Microorganisms	MIC values (mg/ml)						
	Extracts				essential oil (mg/ml)	Control	
	Aq	E	A	C		G	C
S. aureus	0.78	0.097	1.56	>3.12	0.097	0.006	0.048
C. diphtheria	3.12	0.195	3.12	>3.12	0.097	0.012	0.006
E. coli	>3.12	0.78	>3.12	>3.12	0.78	0.097	0.006
P. mirabilis	>3.12	3.12	>3.12	>3.12	0.195	0.097	0.006
K. pneumonia	>3.12	1.56	>3.12	>3.12	0.195	0.048	0.006
S. typhi	1.56	0.195	1.56	>3.12	0.78	0.006	0.006
P. aeruginosa	0.195	0.78	1.56	>3.12	0.048	0.006	0.097

Abbreviations: Aq: Aqueous, E: Ethanol, A: Acetone, C: Chloroform, G: Gentamycin, C: Cefalexin.

DISCUSSION:

Mastic gum has attracted much attention as a natural useful substance in folk medicine to treat a variety of ailments for its antibacterial, anti-inflammatory and anti-ulcer activities[19,22,34].Because of this broad spectrum of biological activities, mastic gum has attracted much attention as a natural useful substance in medicine, health food, and cosmetic industries. These results support the possibility that mastic gum also has potential antibacterial activity against oral bacteria. In the study of Magiatis *et al*.,[23] the in vitro antimicrobial activity of the three essential oils of *P. lentiscus* and of the resin (total, acid and neutral fraction) against six bacteria and three fungi was reported. Two studies from Iraq have suggested that mastic may be effective for ulcer treatment, but one was uncontrolled[19] and the other seriously flawed.

We found that the antimicrobial activity of the mastic gum varied depending on the sample, dosage of mastic gum, and the solvent used (i.e., aqueous , chloroform, ethanol, or acetone). The inhibitory effect of the mastic gum has been increased with increasing mastic gum concentration**.** The diameters of the maximum inhibitory zones at the highest mastic gum dosage (50 mg/ ml) for the aqueous and ethanol extracts. Although mastic gum dissolved in all four of the solvents (chloroform, ethanol, acetone and aqueous) had a good antimicrobial activity against *S. aureus*, mastic gum dissolved in ethanol was found to be the most effective. Compared with the antibiotic (gentamycin; as a control drug) tested, mastic gum had a similar antimicrobial activity against *P. aeruginosa*.

The chemical composition of mastic gum is: l-3% essential oil, 4% a- and b-mastichinic acid (C23H3604), 0.5% masticholic acid (C2sHs604), 20% a-mastichonic acid (C2sH4s04), 18% b-mastichonic acid (Cs2H4sO& 30% a-mastichorezene (C35H5604)and20%b-mastichorezene(Cs5H5604)[35].

The results obtained in this study are in agreement with other investigations in that Gram positive bacteria were more sensitive to essential oils than Gram negatives [36] ,although Deans and Ritchie [37] found that there was no evidence of degree of susceptibility to various essential oils being reflected in the Gram reaction of the organism: Gram positive and negative bacteria were both susceptible. Generally the extent of the inhibitory activity of the essential oils could be attributed to the presence of aromatic nucleus containing a polar functional group [38]. Additional in vivo studies and clinical trial would be needed to justify and further evaluate the potential of these extracts as antibacterial agent in topical or oral application.

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التأثير التثبيطي للمستخلصات والزيت الأساسي المفصول من صمغ نبات المستكي *Pistacia lentiscus* L. ضد عدد من البكتريا المرضية

حسن فيصل حسين المولى

قسم علوم الحياة ، كلية التربية ، جامعة الموصل ، الموصل ، العراق

***E**-mail: hassssanbac@gmail.com*.

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الملخص:

تم في هذه الدراسة التحري عن التأثير التثبيطي للمستخلص المائي و الايثانولي و الاسيتوني و الكلوروفورمي لصمغ نبات المستكي *Pistacia lentiscus* ضد سبعة أنواع مختلفة من البكتريا المرضية: *Staphylococcus aureus, Corynebacterium diphtheria, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas aeruginosa* بأستخدام طريقة microdilution assay. جميع المستخلصات أظهرت تأثيرا تثبيطيا ضد جميع أنواع البكتريا قيد الدراسة ماعدا *E. coli* و *P. mirabilis* اللذان قاوما المستخلص الاسيتوني و الكلوروفورمي.اظهر المستخلص الايثانولي تأثيرا تثبيطيا عاليا بينما اظهر المستخلص المائي تأثيرا اقل في حين أن المستخلص الاسيتوني و الكلوروفورمي تباينا في التأثير بين التأثير الضئيل وعدمه. اظهر الزيت الأساسي المفصول تأثيرا تثبيطيا جيدا ضد جميع أنواع البكتريا قيد الدراسة وبقطر تثبيط تراوح بين (18-27 mm). كما وجد أن التركيز المثبط الأدنى للمستخلص الايثانولي اظهر نتائج جيدة ضد جميع أنواع البكتريا قيد الدراسة وكان بكتريا *S.aureus* أكثر الأنواع البكتيرية حساسية وبتركيز مثبط أدنى(0.097 mg/ml) في حين أن اقل حساسية أظهرت من قبل بكتريا *C. diphtheria* وذلك باستخدام المستخلص الاسيتوني والمائي.وأخيرا اظهر الزيت الأساسي نتائج جيدة ضد بكتريا *P. aeruginosa* كما وأظهرت النتائج اقل تأثير ضد بكتريا *E. coli* و *S. typhi* .