The Antibacterial activity of Pistacia khinjuk resionous exudate

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Abstract

A crude resinous exudate from *Pistacia lentiscus* related *Pistacia khinjuk* tree distributed in Kurdistan mountains has been used focally in curing some lesions including burns. The preparation solved in 50% ethanol and diluted by distilled water was appeared to have antibacterial activity. The minimum inhibitory concentration (MIC) of the resin by well diffusion agar for *Staphylococcus aureus* was 50 µg/ml and with no effect on *P. aeruginosa*. By dilution broth method, MIC was 125 µg/ml for *S. aureus* and 500 µg/ml for *P. aeruginosa* .The killing effect against *P. aeruginosa* occurs also by the solvent 50% ethanol which revealed that resin has no effect on this species. The minimum bactericidal concentration was the same of MIC for both species. The effect of resin was not belonged to phenol only that the MIC of phenol by well diffusion agar was lower than resin for *S. aureus*.

Keywords: *Pistacia khinjuk*, *Pistacia lentiscus*, mastic, resin, antibacterial activity, bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Introduction

The mastic tree *Pistacia khinjuk* is an evergreen bush belongs to the family Anacaradiaceae, it is widely distributed in the Kurdistan region of Iraq, its height is about 3-7m[1]. *P. khinjuk* stems excrete an exudate whenever it cut, this exudate has a common Kurdish synonym (beneshta tal; gum) and used as focal therapy to treat burns and stomach ulcer. The exudate is looking like that of the Mediterranean related species *Pistacia lentiscus* which some work was done on it. The resinous exudate is concentrated to form mastic gum has been used as food flavor and shown to have therapeutic effect against some lesions. In Iraq, *P. lentiscus* and *P. khinjuk* mastic has much used to flavor the locally produced date-spirit (Araq) commonly known as

mastaki[1]. It has been documented as food preservative in ancient works of Greece, Rroman and Arabian physicians' authers[2], it has antioxidant properties[3]. The most literatures have been related with mastic rather than preconcentration resinous exudate. Mastic has been used in Spain as cure for hypertension[4], healer for the relief of upper abdominal discomfort, gastralgia, dyspepsia, peptic ulcer[5, 6] and duodenal ulcer healing[7]. The *in vitro* antimicrobial activity of the whole plant extract of *P. lentiscus* against bacteria and fungi has been reported to be effective on *Sarcina lutea, Escherichia coli, Candida albicans, Candida parapsilosis, Tarulopsis glabrata* and *Cryptococcus naotomonae*[8]. The essential oils of *P. lentiscus* has been shown to be effective on two gram-positive bacteria *Staphylococcus aureas* and *Staphylococcus epidermidis,* four gram-negative bacteria *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as well as the pathogenic fungi *Candida albicans, Candida tropicals* and *Torulopsis glabrata* [9] and *Trichophyton mentagrophytes*[10]. This study aimed to investigate the minimum inhibitory and bactericidal concentrations of *P. khinjuk* resin for two pathogenic bacterial isolates *Staphylococcus aureas* and *Pseudomonas aeruginosa.*

Materials and Methods

Test organism: Two pathogenic isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from burn cases of inpatients in Sulaimani Teaching Hospital.

A mature tree in Hawraman district mountains was identified as *Pistacia khinjuk* according to "The Flora Of Iraq"[1]. Cut of a few millimeters into the trunk of the tree was made in July 2001 starting from the lower parts upwards. This process was done at weekly intervals over 5 to 6 weeks. The resin was collected aseptically in clean dark glass containers with wide opening. Containers were preserved in refrigerator. A stock solution of resin (500 µg/ml) was prepared in 50% ethanol with shaking in water bath at 60°C for two minutes till homogeneous solution was obtained. Final serial dilutions were prepared in distilled water; the preparations were sterilized by filtration through 0.45 Millipore filter. Equivalent concentrations of phenol were prepared in distilled water. The resin and phenol concentrations were used to determine the

minimum inhibitory concentration (MIC) quantitatively by a well diffusion agar method derived from the agar diffusion method employed for the estimation of penicillin in plasma[11], also the MIC and the minimum bactericidal concentration (MBC) were determined by dilution broth method[12]. The well diffusion method was done using Muller-Hentone agar (oxoid) poured in duplicated sterile Petri dishes (9.0cm diameter) were made in agar then seeded by heavily streaking in all directions of the test organism. Wells (5mm diameter), 50µl of the resin was added to each well, the same was done with phenol preparation. Also 50µl of pure undiluted resin were applied. All plates were incubated at 37°C for 18 h.; the inhibition zone was measured as the mean radii diameter in millimeters of clear zone around wells. The minimum inhibitory concentration was identified as the lowest concentration showed clear zone. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration

(MBC) were also measured for the two test organisms according to the NCCLS reference method[12] which described by Iauk [8]. A100µl of Isosensitest broth (oxoid) was added to the first well of microdilution plate and the serial twofold dilutions from the stock solution were performed discarding the final 100µl. The last well contained broth with no drug used as control. A suspension organism grown for 24 h. at 37°C (1µl) was added to each well containing the drug and to the control well. The final concentration of each microorganism in every well was 1x 10^5 CFU/ml. Plates were sealed with transparent acetate and incubated at 37°C under atmospheric conditions for up to 18 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that inhibited visible growth after 18 h. The minimum bactericidal concentration (MBC) was determined by monitoring the subculture of the tubes showing no growth on plates of Brain-Heart-infusion agar (BHIA) without drug. The (MBC) was defined as the lowest concentration of drug that inhibited 99.9% of the growth in subcultures.

Results

By well diffusion agar, the minimum inhibitory concentration (MIC) of the resin and phenol has been shown in (table 1). For *S. aureus*, resin was showed to have MIC at

50 µg/ml while that of phenol was 110 µg/ml. The lowest concentration of resin showed inhibition but the higher ones (110-250 µg/ml) were with no killing effect. For *P. aeruginosa,* resin shown no effect within all concentrations used (50-500 µg/ml) whereas phenol seen to have MIC at 120 µg/ml.

Table 1: The minimum inhibitory concentration (MIC) of phenol and *Pistacia khinjuk* resin for *S. aureus* and *Psidomonas aeruginosa* by well diffusion agar.

Phenol concentration (µg/ml)	Inhibition zone (mm)		Resin concentration (µg/ml)	Inhibition zone (mm)	
	S. aureus	*P. aeruginosa*		*S. aureus*	*P. aeruginosa*
50	5.0	5.0	50	8.0	5.0
100	5.0	5.0	100	6.0	5.0
110	30.0	5.0	110	5.0	5.0
120	13.5	28.0	120	5.0	5.0
130	29.5	30.0	130	5.0	5.0
140	30.5	31.0	140	5.0	5.0
150	32.5	31.0	150	5.0	5.0
200	34.0	33.0	200	5.0	5.0
250	35.0	35.0	250	5.0	5.0
500	36.0	35.0	500	6.0	5.0

By dilution broth method (table 2), resin showed MIC for *S. aureus* at 125 µg/ml, this concentration contained 12.5 % ethnol as solvent which was not the MIC for *S. aureus*. The MIC of ethanol for *S. aureus* was 25 %. The MIC of resin for *P. aeruginosa* was 500 µg/ml, the concentration which contained 50 % ethanol which in turn appeared to be the MIC for *P. aeruginosa*. On subculturing the tubes on Brain-Heart Infusion Agar without drug, the MBCs of both ethanol and resin for two test organisms were the same of MIC that tubes showed no visible growth were shown to have no growth also on BHIA.

Table 2: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of P. *khinjuk* resin and its solvent (ethanol) for *S. aureus* and *P. aeruginosa* using the dilution method of NCCLS, 1992.

Ethanol %	*Viable CFU/ml on BHIA		Resin concentration (µg/ml)	Viable CFU/ml on BHIA	
	S. aureus	*P. aeruginosa*		*S. aureus*	*P. aeruginosa*
**control	0.5×10^6	0.7×10^6	**control	0.5×10^6	0.7×10^6
0.77	42×10^3	45×10^3	7.7	15.7×10^3	20.2×10^3
1.56	33×10^3	44×10^3	15.6	13.5×10^3	18.0×10^3
3.125	1.0×10^3	2.0×10^3	31.25	14.3×10^3	18.5×10^3
6.25	0.15×10^3	2.0×10^3	62.5	5.1×10^3	17.0×10^3
12.5	0.05×10^3	1.0×10^3	125	0.0	20.0×10^2
25	0.0	0.3×10^3	250	0.0	15.5×10^2
50	0.0	0.0	500	0.0	0.0

*: The count was achieved for all tubes included in MIC test.
**: Control for both treatments (ethanol and resin) is the broth with no drug.

Discussion

The parts of *Pistacia spp.* especially *P. lenticus* have been used focally in remedy for some lesions. The aerial part has traditionally been used in the Mediterranean area of Spain as a popular cure for hypertension and the lyophilized aqueous extract produced definite hypotensive effect in anaesthetized normotensive rates[4]. The mastic of *Pistacia* has also been used in curing different lesions of which is burns in our country, for this reason we used *S. aureus* and *P. aeruginosa* as model organisms due to their participation in burn inflammations.

It was appeared that resinous exudate of *P. khinjuk* has antibacterial activity against *S. aureus* but not *P. aeruginosa* by the two tests used, well diffusion agar and dilution broth method. The aerial part extracts have been reported to have activity in different levels similar to those of other vegetable extr... such as the ajoene fraction of *Allium sativ.* (garlic) [13]. It was active on bacteria except for *S. aureus*[8]. Total Resin, as mastic, has been appeared to have antibacterial activity (bacteristatic) against several bacterial species. It was reported to be active on *S. aureus* with a similar activity for both neutral and acidic extracts, but on *P. aeruginosa* the acidic extract was the active one in spite of that it was less active against *P. aeruginosa* than *S. aureus* [9]. Also the same investigators revealed that the MIC of *P.*

lentiscus resin for *S. aureus* is 2.25 mg/ml and *P. aeruginosa* 4.50 mg/ml by using the dilution technique for resin distillated for 3 hours. This study revealed a less MIC values for *S. aureus* (125 µg/ml; table, 2) by dissolving resin in 50% ethanol and the effect was belonged to resin rather than ethanol that the dilution represented the MIC concentration contained sublethal effect of ethanol. The effect of resin was with bactericidal effect at the concentrations used *in vitro*, so that the crude preparation of resin is with value when applied *in vivo*.

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چالاکی دژە بەکتیریای هاوێشتە بنێشتەیی رووەکی*Pistacia khijuk*

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پوختە

دەرهاوێشتە بنێشتەیی(بنێشتە تاڵ) خاوی رووەکی*Pistacia khijuk* کە پەیوەندییەکی نزیکی هەیە بە رووەکی *Pistacia lentiscus* بە کارهێنراوە بۆ تیمارکردنی چەند ئافاتێك بەشێوەیەکی کەلەپووری لەوانە سووتاوی. بنێشتە تاڵ کە بە تواندن لە ٥٠٪ ئیسانۆڵ ئامادە کراوەو بە ئاوی هەڵمێنراو شل کراوەتەوە دەرکەوت کە چالاکی دژی بەکتیریای هەبوو. کەمترین پەیتی وەستێنەر(MIC) بەرێگای ئاگاری تەشەنەی چاڵ بۆ بەکتیریای*Staphylococcus aureus* دەرکەوت ٥٠ مایکرۆگرام/مل بەڵام بێ ئەوەی کاریگەری هەبێت لەسەر بەکتیریای *Pseudomonas aeruginosa* . بەرێگای رووبەرنکردنەوەی شلە، کەمترین پەیتی وەستێنەر بۆ *S. aureu*١٢٥ مایکرۆگرام/مل بوو بەڵام بۆ*P. aeruginosa* ٥٠٠ مایکرۆگرام/مل بوو. کاریگەری کوشندەی بنێشتە تاڵ لەدژی *P. aeruginosa* گەرایەوە بۆ تویننەر، ٥٠٪ ئیسانۆڵ، لەبەر ئەوەش دەرئەکەوێت کە بنێشت هیچ کاریگەریەکی نیە لەسەر ئەم جۆرە بەکتیریایە. دەرکەوت کە کەمترین پەیتی کوشندە (MBC) بۆ هەردوو جۆرە بەکتیریاکە هەر خۆیەتی (MIC) یە بەڵام کاریگەری بنێشتە تاڵ تەنها بۆ فینۆڵ ناگەرێتەوە چونکە کەمترین پەیتی وەستێنەر بۆ فینۆڵ لە بنێشتەکە کەمتر بوو.

الفعالية الضد بكتيرية للراشح الهلامي لنبات Pistacia khijuk

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الخلاصة

يستخدم الراشح الهلامي الخام لنبات *Pistacia khijuk* ذو العلاقة الوثيقة بنبات *Pistacia lentiscus* المنتشر في جبال كردستان في علاج بعض الآفات و من ضمنها الحروق بشكل تقليدي . لقد تبين بأن التحضير المذاب في ٥٠٪ ايثانول و المخفف بالماء المقطرذو فعالية ضد بكتيرية. كان التركيز المثبط الادنى(MIC) للهلام بطريقة غراء انتشار الحفرة ٥٠ مايكروغرام/مل لبكتريا *Staphylococcus aureus* بينما لم يكن له تأثير يذكر على بكتريا *Pseudomonas aeruginosa* . كما تبين بطريقة التخفيف بالمرق بأن التركيز المثبط الادنىلبكتريا *S. aureus* كان ١٢٥ مايكروغرام/مل و لبكتريا *P. aeruginosa* كان ٥٠٠ مايكروغرام/مل .لقد عاد التأثير القاتل ضد بكتريا *P. aeruginosa* الى المذيب،٥٠ايثانول، أيضا والذي يؤكد بأن الهلام ليس له تأثير على هذا النوع البكتيري. لقد كان التركيز المبيد الادنى (MBC) هو نفسه(MIC) لكلا النوعين البكتيريين. لم يكن التأثير الضد بكتيري للهلام عائدا الى الفينول فقط ، ذلك ان التركيز المثبط الادنى للفينول و بطريقة غراء انتشار الحفرة كان أقل من الهلام لبكتريا *S. aureus*.

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