IN *VITRO* ANTIBACTERIAL ACTIVITY OF *PISTACIA LENTISCUS* L. (Misteka) AGAINST SOME HUMAN BACTERIAL INFECTIONS.

By

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ABSTRACT

The volatile oil , gum and resin ethanolic extracts of *Pistacia lentiscus* L (Misteka) were tested for a preliminary antibacterial screening against one Gram positive organism(*Staphylococcus aureus*) and three Gram negative organisms (*Escherichia coli* ,*Proteus vulgaris* and *Pseudomonas aeruginosa*). All extracts exhibited high antibacterial activity against the organisms tested. Therefore they were further tested against 14 clinical specimens isolated from different sources (abscess, urinary tract infection and wounds specimens).The standard bacteria were tested against two antibiotics and the results were compared with the activity of the plant extracts.

الملخص

تم اختبار مستخلصات الصمغ، الزيت والراتنجلنبات المصطكى بالإيثانول ضد نوع واحد من البكتريا موجبة الجرام وهي (العنقودية الذهبية) وثلاثة أنواع من البكتريا سالبة الجرام (الإشريكية القولونية والزائفة الزنجارية والمتقلبة الإعتيادية). وقد أظهرت أن جميع الخلاصات لها فاعلية عالية ضد جميع أنواع البكتريا المختبرة.

كما تم اختبار الخلاصات أعلاه ضد 14 عزلة اكلينيكية من البكتريا معزولة من مرضى من مصادر مختلفة(خراج وخمج المجاري البولية والجروح).

تم إختبار الكائنات المعيارية ضد مضادات حيوية مرجعية وتمت مقارنة النتائج مع فاعلية الخلاصات.

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INTRODUCTION

Pistacia lentiscus L (Mastic gum tree) locally known as Misteka belongs to the family Anacardiaceae .It is a small tree or shrub more than 12 feet in height with parpinnate leaves and lanceolate to obviate lanceolate leaflets. The inflorescence is compact and spike- like, flowers are yellowish or purplish and the fruit is a drupe [1]. The mastic product originates in a special oleoresin reservoirs located in the inner bark of the trunk and branches[2]. The tree is widely spread in countries bordering the Mediterranean Sea. From there the tree was exported to Great Britain (2). Oil of the mastic is a colourless liquid with a pronounced balsamic odor. Resin is obtained after removal of oil by water distillation. The crude mastic is a gummy substance[3].

The chemical composition of the essential oils from the gum and from various parts of *Pistacia lentiscus* L are α- pinene, ß-myrcene, α- terpineol, terpinen-4-ol and Dimyrcene (4 isomers) [4].

Resinous exudation of tree is known as Mastic,, and is employed as analgesic and sedative in gastralgia cardiodynia, mastitis, peptic ulcer, boils, carbuncles, also as antitussive and expectorant. Resin is prescribed as a nervous calmative (infusion in tea) and emmenagogue, chewed to purify the breath, enters in cosmetic products and in depilatory cream. Resin boiled with milk is used for throat troubles and for ulcers. A teaspoonful of mastic pounded and mixed with an equal amount of honey is taken first thing in the morning for three weeks. Decoction of root is used for cough. Dried and fumigated bark of the trunk and branches is applied to facilitate childbirth. Infusions of leaves are diuretic, astringent, emmenagogue. A peeled nut yields oil by hot extraction which is effective against itch and by rubbing for treating rheumatism[5]. Locally mastic with Sesame oil are used for treatment of cough and respiration distress in children (Altiaman , Personal communication, 2000).Therefore the main objective of this preliminary study was to run screening tests using various extracts of *Pistacia lentiscus* L. to demonstrate their antimicrobial activity against different pathogenic bacteria, both standard and clinical specimens. Furthermore, the antimicrobial activity obtained with two standard antibiotics routinely used against human bacterial infections.

MATERIALS AND METHODS

Mastic gum was collected from Altaiman Shopping Center at Omdurman, Sudan..Wail Alsadic Abdalla identified it. (Medicinal and Aromatic Plants Research Institute), National Centre for Research., Khartoum Sudan..

Method of Extraction: The oil of mastic was obtained by water distillation technique using Clevenger method according to Guenther[6]. Four hundred and fourty grams of powdered mastic were placed in a 2-litre round bottom flask, and distilled water was added and mixed thoroughly. The contents of the flask were boiled gently for four hours until the volatile oil has been distilled. The crude volatile oil (4.4ml) was transferred by means of a pipette in a brown bottle. Anhydrous sodium sulphate was added, agitated gently to absorb the water and the clear oil was decanted into a brown bottle and kept in the refrigerator till used. The residue of water distillation was the resin. One gram of it was dissolved in 10ml of 95% ethanol and was tested against the standard and clinical isolates at different concentrations. One gram of Mastic gum was dissolved in 10ml of 95% ethanol.

Studied Activity: The antibacterial activity of the extract was performed using the cup-plate agar diffusion method[7]. with some minor modification to asses the antibacterial activity of the prepared extracts. Two ml of the standardized bacterial stock suspension (10^8-10^9) colony forming units per ml were thoroughly mixed with 200 ml of sterile Molten nutrient agar which was maintained at 45°C.

Twenty ml aliquots of the inoculated agar were distributed into sterile petri-dishes. The agar was left to set and each of these plates 4 cups (10mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each of the extracts using adjustable pipette, and allowed to diffuse at room temp for two hours. The plates were then incubated in the upright position at 37°C for 18 hours; two replicates were carried out for each extract against each of the tested organisms. After incubation, the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Microorganisms: Standard organisms *Staphylococcus aureus* NCTC 6447, *Escherichia coli* NCTC8196, *Proteus vulgaris* ATCC6380 and *Pseudomonas aeruginosa* NCTC6750 obtained from the Department of Microbiology, Faculty of Pharmacy, University of Khartoum and the clinical isolates (n = 14) were isolated from various clinical specimens from Sudanese patients.

RESULTS AND DISCUSSION

Different concentrations of Pistacia extracts (oil, gum and resin) and two types of antibiotics (Ampicillin and Gentamicin), were tested against four standard bacteria (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*).

Results presented in (Table 1) indicated that the antibiotics showed relatively high antibacterial activity against standard species of bacteria compared to mastic extracts except for *Escherichia coli* and *Pseudomonas aeruginosa* which were not affected by ampicillin.

Gentamicin exhibited the highest antibacterial activity against all Gram-negative bacteria, but its effect in Gram-positive *Staphylococcus aureus* was relatively low. Ampicillin had higher antibacterial activity against *Staphylococcus aureus,* and *Proteus vulgaris*, whereas the other organisms did not respond to its application.

Mastic extracts showed moderate antibacterial activity against standard bacteria compared to the antibiotics (Table1). The mastic extracts showed relatively high antibacterial activity against *Escherichia coli*.

In fact mastic was used with sesames oil locally as antiseptic for treatment of cough and respiration distress in children and it proved to be effective in curing that disease. The observed antibacterial activity of the various extracts might justify its traditional use.

Effect of Mastic on *Escherichia coli:* (Table 2) showed the values of antibacterial activity of mastic extracts on *Escherichia coli* (clinical isolates and standard organi-sms). The results indicated that the growth of standard *E- coli* was significantly ($P < 0.05$) reduced (18.5) compared to that clinical isolates (16.6mm). The species isolated from wound exhibited the lowest activity relative to other clinical isolates. This may be attributed to the fact that some isolated

bacteria have a resistance developed by the individual of the subject which might have used different types of antibiotics.

Table (1): Screening of Antibacterial Activity of *Pistacia lentiscus* L. Against The Standard Organisms

Family/Botanical name/Vernacular name	Part Used	Solvent Used	Concentration Used	Test Organisms Used* M.D. I.Z.** mm			
				E.c	*Pr.v*	*Ps.a*	*S.a*
Cardiaceae *Pistacia lentisccus* L **Ver. Name** Misteka	Gum	EtOH	1(mg/m1)	18	16	16	16
			2(mg/m1)	20	18	18	17
			3(mg/m1)	24	20	20	17
	Oil	EtOH	0.10(v/v)	16	16	16	15
			0.20(v/v)	18	17	18	17
			0.30(v/v)	18	20	23	22
	Resin	EtOH	0.1(mg/m1)	16	16	16	16
			0.2(mg/m1)	16	17	16	18
			0.3(mg/m1)	20	20	20	20
Reference Drugs	**Concentration Used (mg/m1)**			*E.c*	*Pr.v*	*Ps.a*	*S.a*
Ampicillin	0.005			-	-	-	18
	0.01			-	17	-	20
	0.02			-	20	-	25
	0.04			-	25	-	26
Gentamicin	0.005			12	15	-	13
	0.01			16	16	15	14
	0.02			19	20	21	16
	0.04			24	25	22	17

E.c* = *Escherichia coli*, Pr.v = *Proteus vulgairs*, Ps.a = *Pseudomonas aeruginosa*, Sa = *Staphylococcus aureus*, M.D.I.Z** = Mininmum diameter of growth inhibition zone in mm.
Average of two replicates, - = No inhibition.

Oil extracts gave relatively better control results for the various bacterial species compared to other extracts, but considering concentrations, the other extracts may perform better for the same concentration. Generally the antibacterial activity of the various extracts increased slightly with increasing concentration. The antibacterial effect of oil extracts on *E. coli* (urinary isolates) was low on E. C.1 compared to E.C. 4, whereas the effect of other extract on the same clinical isolates was almost similar. This discrepancy is not justifiable and experimental errors can not be excluded.

Effect of Mastic on *Proteus vulgaris*: Antibacterial activity of mastic extracts on *Proteus vulgaris* (clinical isolates and standard strains), indicated that the inhibition zone of clinical isolates was relatively

low[6] compared to standard one (17.8mm). Prot. V3 (urinary isolates) showed the highest antibacterial activity (18mm) compared to all other species. It seemed striking that Prot. V1 was a urinary isolates also, but it gave low antibacterial activity (15.5mm), this may be explained by the fact that these isolates were from different subjects and such differences were expected.

Mastic gum extracts showed slightly high antibacterial activity (16.5mm) compared to oil and resin which gave (15.5mm) and (16mm), antibacterial activity respectively (Table 3). The antibacterial activity increased significantly ($P < 0.05$) with increasing concentra-tion of the various mastic extracts. Generally these results were in agreement to the previous findings, which reported clearly that Mastic has moderate antibacterial activity against most of the organisms examined[8].

Table (2): Antibacterial Activity of *Pistacia lentiscus* L. Against The Standard and The Clinical Isolates of *Escherichia coli*.

Extract	Concentration Used	Mean Diameter Of Inhibitory Zone (mm)*					
		E.c1	E.c2	E.c3	E.c4	Mean	E.c.std
Volatile Oil	1(v/v)	18	18	13	16	16.3	18
	2(v/v)	20	20	14	16	16.5	20
	3(v/v)	22	22	16	16	19	24
Mastic Gum	0.1(mg/ml)	15	15	14	16	15	16
	0.2(mg/ml)	16	17	17	16	16.7	18
	0.3(mg/ml)	18	18	18	18	18	18
Resin	0.1(mg/ml)	14	15	14	16	14.7	16
	0.2(mg/ml)	16	16	16	16	16	16
	0.3(mg/ml)	18	18	17	17	17.5	20

Area of isolation: **E.c1** Urinary tract, **E.c2** = Ear swab, **E.c3** = Wound, **E.c4** = Urinary tract, **E.c.std** = Standard E. c, * Average of two replicates, 15 mm Inhibitory zone = Sensitive, less Than 15 mm = Resistant.

Effect of Mastic on Pseudomonas Aerations: Data displayed in (Table 5) showed that the antibacterial activity of mastic extract on *Pseudomonas aeruginosa* (clinical isolates and standard species) was moderate. Mean inhibition zone of standard species was higher than the clinical isolates. The wound isolates of *Pseudomonas aeruginosa* showed relatively high susceptibility to mastic extracts (16.6mm) compared to urinary tract isolates (15.7mm). Mastic gum showed higher antibacterial activity compared to other extract (Table 4).

Generally the antibacterial activity of the various extracts against *Pseudomonas aeruginosa* increased significantly ($P < 0.05$) with increasing concentration.

Table (3): Antibacterial Activity of *Pistacia lentiscus* L. Against The Standard and The Clinical Isolates of *Proteus vulgaris*

Extract	Concentration Used	Mean Diameter Of Inhibition Zone in (mm)*					
		Pr.v1	*Pr.v2*	*Pr.v3*	*Pr.v4*	*Mean*	*Pr.v.std*
Volatile Oil	1(v/v)	18	18	13	16	16.3	18
	2(v/v)	20	20	14	16	16.5	20
	3(v/v)	22	22	16	16	19	24
Mastic Gum	0.1(mg/m1)	15	15	14	16	15	16
	0.2(mg/m1)	16	17	17	16	16.7	18
	0.3(mg/m1)	18	18	18	18	18	18
Resin	0.1(mg/m1)	14	15	14	16	14.7	16
	0.2(mg/m1)	16	16	16	16	16	16
	0.3(mg/m1)	18	18	17	17	17.5	20

Area of isolation: *Pr.v1* = Urinary tract, *Pr.v3* = Urinary tract, *Pr.v4* = Ear swab, *Pr.v.std* = Standard
* Average of two replicates, 15 mm Inhibitory zone = Sensitive, less Than 15 mm = Resistant

Table (4): Antibacterial Activity Of *Pistacia lentiscus* L. Against The Standard And The Clinical Isolates Of *Pseudomonas aeruginosa*

Extract	Concentration Used	Mean Diameter Of Inhibition Zone in (mm)*				
		Ps.a1	*Ps.a2*	*Ps.a3*	*Mean*	*Ps.a.std*
Volatile Oil	1(v/v)	13	16	14	14.3	16
	2(v/v)	14	16	14	14.7	18
	3(v/v)	18	18	15	17	20
Mastic Gum	0.1(mg/m1)	16	16	16	16	16
	0.2(mg/m1)	16	17	16	16.3	18
	0.3(mg/m1)	18	20	18	18.7	18
Resin	0.1(mg/m1)	15	15	16	15.3	16
	0.2(mg/m1)	15	15	16	15.3	17
	0.3(mg/m1)	16	16	17	16	17
		15.3	15.3	16.37	15.3	16.3

Area of isolation: *Ps.a1* = Urinary tract, *Ps.a2* = Wound, *Ps.a3*= Urinary tract, *Ps.a.std* = Standard *Ps.a*
* Average of two replicates, 15 mm Inhibitory zone = Sensitive, less Than 15 mm = Resistant

Table (5): Antibacterial Activity of *Pistacia lentiscus* L. Against The Standard and The Clinical Isolates of *Staphylococcus aureus.*

Extract	Concentration Used	Mean Diameter Of Growth Inhibition Zone In mm.*				
		S.a1	S.a2	S.a3	Mean	S.a.std
Volatile Oil	1(v/v)	14	14	14	16	16
	2(v/v)	14	14	14	14.3	17
	3(v/v)	15	16	15	15.3	17
Mastic Gum	0.1(mg/m1)	15	15	15	15	15
	0.2(mg/m1)	16	17	17	17	17
	0.3(mg/m1)	17	18	19	18	22
Resin	0.1(mg/m1)	15	15	14	14.7	16
	0.2(mg/m1)	16	18	16	16.7	18
	0.3(mg/m1)	17	19	17	17.7	20

Area of isolation: S.a1 = Wound, S.a2 = Urinary tract, S.a3 = Wound, S.astd. = Standard S.a.
* Average of two replicates, 15mm Inhibitory zone = Sensitives, less than 15mm = Resistant.

Effect of Mastic on *Staphylococcus aureus:* Antibacterial activity of mastic extracts on *Staphylococcus aureus* (clinical isolates and standard

strain) was given in (Table 5). The standard species showed the highest inhibition zone (17m) in response to mastic extracts compared to clinical isolates (15.3mm). Generally, the antibacterial activity increased with increasing concentration of the various extracts.

It is worth mentioning that the medium antibacterial activity observed for mastic extracts against Gram positive *Staphylococcus aureus* was of special important, hence the antibacterial activity of mastic was not restricted against Gram negative bacteria. These results were in agreement to the previous findings which stated that mastic had a wide spectrum of antibacterial activity[1 , 8].

Therefore, from the findings of this study, it could be concluded that various extracts of Pistacia L (Misteka) were fairly effective as natural antibacterial agents against various human bacterial infections in comparison to routinely used antibiotics. Although further in-depth studies are needed to clarify this preliminary finding. it could be recommended however, with some reserveations, the use of these natural antibacterial against some common human local (e. g. wounds) or systemic (e. g. ear and urinary tract) bacterial infections.

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