Antibacterial Activity of Some Plant Extracts Against Some Bacterial Species

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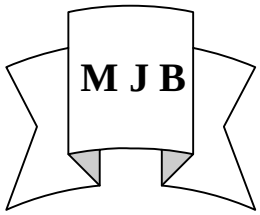

Abstract

Antibacterial activity of some local plant extracts: Cumin (*Cuminum cyminum*), harmal (*Peganum harmala*) and pomegranate (*Punica granatum*)were determined against some bacterial species(*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi and Proteus mirabilis*). These extracts showed variation in their inhibitory effects, the pomegranate*(Punica granatum)*extract revealed the highest inhibitory activity against most bacterial isolates.

The minimal inhibitory concentration(MIC)was also varied with the type of plant extract and bacteria. The MIC of the cumin(*Cuminum cyminum*)was 2.5 gm/dl for *E.coli*, *K.pneumonia* and *S.typhi*. The MIC of harmal(*Peganum harmala*)was 5gm/dl for *K.pneumonia*, while the MIC of pomegranate (*Punica granatum*) was 2.5 gm/dl for *E.coli* and 5gm/dl for the other isolates.

الخلاصة

جرى تحديد الفعالية التضادية لمجموعة من النباتات العراقية الشائعة وهي:الكمون (*Cuminum cyminum*) والحرمل (*Peganum harmala*) و الرمان (*Punica granatum*) تجاه مجموعة من البكتريا المرضية(*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia Salmonella typhi and Proteus mirabilis*) ، حيث أظهرت هذه المستخلصات إختلافاً في فعاليتها التثبيطية، وقد وجد إن مستخلص الرمان كانت له الفعالية التثبيطية الأكبر تجاه أغلب العزلات البكتيرية.

تم قياس التركيز المثبط الأدنى (MIC) لهذه المستخلصات ووجد أن القيمة تختلف باختلاف المستخلص والنوع البكتيري، حيث لوحظ أن التركيز المثبط الأدنى لنبات الكمون كان ٢,٥ غم/العشرل مع كل من بكتريا *E.coli K.pneumonia* و *S.typhi* ،بينما كان التركيز المثبط الأدنى لنبات الحرمل ٥ غم/العشرل مع بكتريا *K.pneumonia* ، أما لنبات الرمان فكان ٢,٥ غم/العشرل مع بكتريا *E.coli* و٥ غم/العشرل مع بقية العزلات الأخرى.

Introduction

Botanical medicines have widely used in treatment of many diseases caused by bacteria , viruses , fungi and other infectious microorganisms in great areas of the world. Although there are wide spectrum of antibiotics for most of the discovered diseases , many people returned to use medicinal plants because they thought to be the most safer and cheaper than synthetic drugs(1). Large number of plants were used in treatment of different diseases because they have one or more compounds that have a role in antimicrobial activity(2).

The effect of pomegranate extracts on bacterial growth was established by many studies on different bacterial species , most of these studies revealed that the pomegranate extracts potentially antibacterial with the ability of endotoxin production inhibition(3). Enterobacteriaceae and other bacterial species have great clinical and epidemiological importance as etiologic agents in human diseases including digestive , respiratory , dermatic and urinary diseases(4).

Although pharmacological factories produce new antibiotic formulas continuously ,

resistance of microorganisms to these antibiotics has increased according to genetic ability of microorganisms to transmit and acquire resistance to drugs(5). Iraqi people returned to herbal medicine in the late 20 years because of the absence of many beneficial drugs , expensive cost of others and the bad economical state of people.

Cumin (*Cuminium cyminum*) was applicated as a medicine plant for treatment of many diseases such as diarrhea , gastric disorders , antiseptic agents and other medical uses . This plant has antimicrobial properties against pathogenic bacteria and fungi, indicated that it is an aromatic herb and has a powerful bactericidal action(6) .

Harmal (*Peganum harmala)* was also a widely used as a medicinal herb, its chemical components especially beta- carbolines has been reported to possess antibacterial and antitumor properties(7,8). The present study aimed to estimate the medical importance of three local herbs on growth of five bacterial species and to compare their inhibitory action with the common antibiotics.

Materials and Methods

Collection and Identification of Plant Materials

Cumin(*Cuminum cyminum*)seeds, harmal (*Peganum harmala*) seeds and pomegranate (*Punica granatum*) cortex were purchased from a local market in Hilla . Identification of plant species was determined by Professor Dr. A. Al-Bermani at herbarium of the Department of Biology , College of Science , University of Babylon ,Hilla –Iraq .

Crude Extraction

Hot and cold water extraction were done(9). Binary dilutions were made for the plants to obtain the concentration 1.25,2.5,5 and 10 gm/dl(10).

Bacterial Isolates

The bacterial species were isolated from diarrhea patients at Hilla surgical hospital ; Hilla , Iraq , and identified(11). The bacterial species studied were *Pseudomonas aeruginosa* ,*Escherichia coli, Klebsiella pneumonia* , *Salmonella typhi* and *Proteus mirabilis* .

Antibiotic Sensitivity Test

Eleven types of antibiotic(Chloramphenicol 30mcg, Erythromycin 15mcg, Penicillin G 10U, Trimethoprim 5mcg, Gentamicin 10mcg, Cloxacillin 1mcg, Nitrofurantoin 300mcg, Streptomycin 10mcg, Ampicillin 10mcg, Cephalexin 30mcg, Tetracycline 30mcg) were tested against the bacterial isolates by using Agar disk diffusion(12). The concentration of bacterial suspension used was 1.5*10^8 cell/ml which is equal to 0.5 McFarland standard .

Plant Extract Sensitivity Test

The antibacterial activity of different plant species were evaluated by agar well diffusion using Mueller Hinton Agar(13)**.** The turbidity of bacterial suspensions were compared with 0.5 McFarland standard , then, the bacterial suspension inoculated into Mueller Hinton agar plates.Four wells(7 mm in diameter) were made in each seeded plate by using cork borer .Fifty micro liter of each plant extract concentration were introduced into a well which then incubated at 37˚C for 24 h. Antibacterial activity was determined by measuring the mean values of inhibition zone diameter for each plant extract.

Minimum Inhibitory Concentration(MIC) Test

Inhibitory effect of each plant extract was determined by making four serial dilutions (2-fold dilutions) starting with tube 1 (10%),tube2(5%),tube3(2.5%) and tube4(1.25%), besides the fifth tube which considered as a control . Constant amount of bacterial suspension was added to all tubes which then incubated at 37˚C for 24 h . Then, the tubes were compared with the control tube to determine the lowest concentration of plant extract that inhibits growth of bacteria.

Results

Experiments showed that the hot and cold extracts of cumin (*Cuminium cyminium*) have no inhibitory role on the growth of *Pseudomonas aeruginosa*; *Escherichia coli; Salmonella typhi* and *Proteus mirabilis* but the cold extract revealed inhibitory action on *Klebsiella pneumonia* especially the concentrations 10 , 5 and 2.5 gm /dl. Exposure of bacterial isolates to harmal (*Peganum harmala*) extract detect that the hot and cold extract had inhibitory action on *Klebsiella pneumonia* and *Proteus mirabilis.* Pomegranate(*Punica granatum*) extract revealed the highest effect on

bacterial growth since the hot extract reduced growth of most of bacterial isolates except the *Klebsiella pneumonia* .

The highest inhibitory action shown on *Proteus mirabilis*(table.1). The minimal inhibition concentration (MIC) of cumin is 2.5 gm /dl for *E. coli , K. pneumonia and S. typhi* where the MIC for the cold extract is 5 gm /dl on *S. typhi* only. The hot extract of harmal revealed that the 5gm / dl is the MIC for *Klebsiella pneumonia* only .Moreover the hot and cold extract of pomegranate show highly inhibitory action and the MIC of the cold extract is 10 gm /dl and 5 gm /dl for hot extract on *Ps.aeruginosa* , while the MIC of the cold and hot extract for *E. coli* are 2.5 gm/dl and 5 gm /dl respectively whereas the MIC of *S. typhi* and *Pr. mirabilis* are 5gm/dl and 10 gm/dl(table.2) .

The application of antibiotics on bacterial isolates showed that the most effective antibiotics on growth of bacteria are ciprofloxacin and nitrofurantoin. *Pseudomonas* and *Salmonella* are sensitive to ciprofloxacin while other isolates showed resistance to all antibiotics . *Escherichia coli* and *Klebsiella pneumonia* were sensitive to nitrofurantoin but the other isolates revealed resistance to that antibiotic(table.3).

Discussion

Scientific studies on herbal medicine as alternative source of therapeutics become more common over the previous few years in most countries because of the increase of microorganisms resistance to antibiotics(14). The present study designed to examine the types of bacteria that cause diarrhea in children and to examine the antibacterial activity of three medicinal plants against these isolates. The medicinal plants showed variation in their antibacterial action ,among the three plants harmal showed promising activity against tested microorganisms. This result is in agreement with reports of several workers(15,16). Hot extract of harmal had the highest inhibitory action against all tested isolates. The crude extract had essential materials that inhibits microbial growth, previous findings on leaves, flowers, roots and stems showed arrange of activity against bacteria and protozoa(17). The most sensitive bacterium to tested plants is *Pseudomonas*, on the other hand slightly inhibition was observed by the use of cumin. Voravuthikunchai *et al.*,2004 reported good antibacterial activity in harmal and pomegranate against *E.coli and Klebsiella* using aqueous extract(18). This study revealed that cumin cold extract showed inhibitory activity on *Klebsiella pneumonia.* Most of the ancient cultures especially Babylonians and Egyptians referred to the importance of cumin. Cumin had a noticeable importance in most human equipments , it used in cakes , bread , cheese , sauces , soups and all types of spice mixtures and it has powerful antimicrobial properties(6). The study of Al-Jedah *et al.*, 2000 reported that cumin (*Cuminium cyminium*) had antibacterial activity against *E.coli* when combined with other spices like coriander, mustard and lemon(19). Sagdic *et al.*, 2003 assayed the inhibitory effect of cumin methanolic extract against *E.coli*(20) . Hot and cold pomegranate extract revealed a noticeable inhibitory activity against most bacterial isolates tested , this may be due to the chemical components of this plant which had broad –spectrum antibiotic compounds such as tannic acid and the presence of some metabolic toxins(15,16). The MIC experiments indicated that *E.coli* and *Kl. pneumonia* are the most affected bacteria by the administration of the three plant extracts while the other isolates revealed resistance . Such resistance could be due to the physiological structures of these bacteria including permeability barrier provided by the cell wall or the membrane accumulation mechanism(21). The resistance of bacterial isolates to the tested antibiotics in this study led us to conclude that these antibiotics have no significant efficiency against these bacteria , this may be due to the development of multiple drug resistance by these isolates(22,23).

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Table 1. Inhibitory effect of cold & hot extract concentrations of the plants on bacterial isolates.

Plants	Extract concentration (gm/dl)		Diameter of inhibition zone (mm) PA	EC	KP	ST	PM
Cumin	10	C	-	-	11	-	-
		H	-	-	-	-	-
	5	C	-	-	9	-	-
		H	-	-	-	-	-
	2.5	C	-	-	9	-	-
		H	-	-	-	-	-
	1.25	C	-	-	-	-	-
		H	-	-	-	-	-
Harmal	10	C	-	-	9	10	10
		H	-	-	11	9	9
	5	C	-	-	-	-	9
		H	-	-	-	-	-
	2.5	C	-	-	8	-	8
		H	-	-	-	-	-
	1.25	C	-	-	-	-	-
		H	-	-	-	-	-
Pomegranate	10	C	17	13	-	14	30
		H	23	19	-	15	30
	5	C	14	11	-	-	25
		H	-	11	-	13	25
	2.5	C	9	10	-	-	24
		H	-	11	-	-	20
	1.25	C	-	8	-	-	22
		H	-	-	-	-	20

C = cold extract; H= hot extract ; (-)= no activity.
PA, *Pseudomonas aeruginosa*; EC, *Escherichia coli*; KP, *Klebsiella pneumonia*; ST, *Salmonella typhi*; PM, *Proteus mirabilis.*

Table 2. Minimal inhibitory concentration of cold & hot extract concentrations of the plants on bacterial isolates.

Plants	Extract concentration (gm/dl)		Bacterial isolates PA	EC	KP	ST	PM
Cumin	10	C	-	-	+	-	-
		H	-	-	-	-	-
	5	C	-	-	-	-	-
		H	-	-	-	-	-
	2.5	C	-	-	-	-	-
		H	-	-	-	-	-
	1.25	C	-	-	-	-	-
		H	-	-	-	-	-
Harmal	10	C	-	-	-	-	-
		H	-	-	+	-	-
	5	C	-	-	-	-	-
		H	-	-	-	-	-
	2.5	C	-	-	-	-	-
		H	-	-	-	-	-
	1.25	C	-	-	-	-	-
		H	-	-	-	-	-
Pomegranate	10	C	+	+	-	+	+
		H	+	+	-	+	+
	5	C	+	+	-	-	+
		H	-	+	-	+	+
	2.5	C	-	+	-	-	+
		H	-	+	-	-	+
	1.25	C	-	-	-	-	+
		H	-	-	-	-	+

C = cold extract; H= hot extract ; (-)= no activity; (+)= active concentration.
PA, *Pseudomonas aeruginosa*; EC, *Escherichia coli*; KP, *Klebsiella pneumonia*; ST, *Salmonella typhi*; PM, *Proteus mirabilis.*

Table 3. Inhibitory effect of some antibiotics against bacterial isolates.

Antibiotic	Disk content	Bacterial isolates				
		PA	EC	KP	ST	PM
Cp	30 mcg	S	R	R	S	R
C	30 mcg	R	R	R	R	R
P	10 U	R	S	R	S	R
E	15 mcg	R	R	R	R	R
TMP	5mcg	R	R	R	R	R
S	10 mcg	R	R	R	R	R
FU	300 mcg	S	S	S	R	R
AM	10 mcg	R	R	R	R	R
CN	10 mcg	R	R	R	R	R
TE	30 mcg	R	R	R	R	R
CX	1 mcg	R	R	R	S	R

PA, *Pseudomonas aeruginosa*; EC, *Escherichia coli*; KP, *Klebsiella pneumonia*; ST, *Salmonella typhi*; PM, *Proteus mirabilis.*

Cp,Cephalexin; C,Chloramphenicol; P,PenicillinG; E,Erythromycin; TMP,Trimethoprim; S,Streptomycin; FU,Nitrofurantoin; AM,Ampicillin; CN,Gentamicin; TE,Tetracycline; CX,Cloxacillin.

S= Sensitive; R= Resistant.