A Study of Antibacterial Activity of Cidar (Zizyphus spina-christi L.) on Bacterial Pathogens isolated from Skin Infections

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الخلاصة:

ان الاسم الشائع لنبات Zizyphus spina–christi في العراق هو النبق او السدر. لقد استخدم جميع اجزاء النبات في الدول العربية كمواد محافظة على صحة الانسان . في هذا التقرير, وجد ان للمستخلص المائي والميثانولي لاوراق وبذور نبات النبق وبتراكيز (25 ,50, 100, و 200 ملغم/سم^3) تأثيرا محبطاً لنمو عدداً من انواع البكتريا المعزولة من بعض المصابين بأضرار جلدية. لقد استخدم التركيز 25 ملغم/سم^3 من كل من المستخلصات المختلفة المذكورة اعلاه ولم يسجل اي تأثير محبط للنمو على جميع البكتريا التي اختبرت. اما اعلى تأثير محبط للنمو (17.67 ملم) فقد كان لمستخلص الاوراق المائي على بكتريا Staphylococcus aureus بينما كان الاقل تأثيراً (7.33 ملم) لنفس المستخلص ضد بكتريا Pseudomonas aeruginosa . وقد وجد ان لمستخلص الاوراق المائي والميثانولي تأثيراً مضاداً لنمو البكتريا افضل من مستخلص البذور المائي والميثانولي. لذلك فقد تؤيد النتائج المتحصلة من هذه الدراسة استخدام كل اجزاء نبات النبق لما تحتويه من مواد طبيعية فعالة ضد المكروبات لأنتاج وتصنيع ادوية جديدة لمعالجة الامراض المعدية .

Abstract:

Zizyphus spina-christi is a scientific name of a plant where its common name is Nabaq in Iraq. All parts of this plant are used by local Arab people to help maintain a healthy lifestyle. In this report, it was found that the aqueous and methanolic extracts of leaves and seeds of Zizyphus spina-christi has inhibitory effects at various concentrations (25, 50, 100, and 200 mg/ml) against five bacterial species Staphylococcus aureus, Pseudomonas aeruginosa , E. coli , Acinetobacter spp. ,and Enterococcus spp. isolated from skin lesions. The 25 mg/ml concentration had no effects at all on all bacterial species tested. The highest activity (17.67 mm) recorded was for leaves aqueous extract against Staphylococcus aureus, while the lowest activity (7.33 mm) was obtained for leaves aqueous extract against Pseudomonas aeruginosa. The leaves aqueous and methanolic extracts had better antimicrobial activities than seed equeous and methanolic extracts. Our results support, at least in part, the use of all parts of

Zizyphus spina-christi plant as a natural antimicrobial agent in developing new drugs for treating infectious diseases in human.

Introduction:

It is well known in medical fields that microorganisms have developed resistance to different antibiotics already in use. This phenomena has created a tremendous clinical problems concerning the treatment of diseases caused by such microorganisms. Therefore, there is a continuous and urgent needs to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action from various sources, such as medicinal plants [1,2] . Antimicrobials of plant origin are efficient in the treatment of infectious diseases mitigating simultaneously many of the side effects that are often associated with synthetic antimicrobials [3,4].

The genus Zizyphus belong to the family Rhamnaceae. It is a genus of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world [5]. Zizyphus spina-christi has been used in folk medicine as a demulcent, depurative, anodyne, emollient, stomachic, for toothaches, astringent and as a mouth wash [6]. This plant has been extensively studied and its chemical composition determined [7], the main constituent were betulic and ceanothic acid [8] and three cyclopeptide alkaloids as well as four saponin glycosides [9]. Several flavonoides have been isolated from the leaves of Zizyphus spina-christi [10]. The oil of the Zizyphus spina-christi has been used for washing the hair and the body [11] and the plant leaves were also used in medicine as an antiseptic, antifungal and anti-inflammatory agent and for healing skin diseases such as atopic dermatitis [12]. The saponin fraction of the leaves has an antimicrobial activity against Candida albicans [13].

One of the possible methodologies that can be used for the discovery of antibacterial active principles is the screening of the selected plant extracts for the activity followed by bioassay-guided fractionation of active extracts leading to the isolation of the pure constituent. Zizyphus spina-christi is a famous plant in Iraq and distributed all over the Iraqi regions especially middle and southern parts. The present work aimed to investigate the effective concentrations of the aqueous and methanolic extracts of leaves and seeds of Zizyphus spina-christi L. that may inhibit the growth of some microorganisms isolated from skin infection in comparison with the standard antibiotic Tetracyclin.

Material and Methods:

Collection of plants:

Leaves and seeds of Zizyphus spina-christi plant used in this study were collected from various regions of Baghdad during February and May of 2009.

Preparation of extracts:

The extraction method of Mashhadian and Pakhshandel [14] was used. The leaves and seeds were dried under shade for about one week and then at 40ºC in

an incubator for 2 – 3 days. The dried leaves and seeds were pulverized with mortar and pestle or electric mill, aqueous and methanolic extraction were performed by weighting 30 grams of fine powder of leaves or seeds and were sucked with 300 ml. each of water or methanol with extraction period of 10 – 12 hrs. This process was repeated three times, and the three aqueous or methanol extracts were combined and were filtered using Whitman filter paper No.1 and the solvents were evaporated using rotary distillation apparatus. In order to obtain a complete dry extracts, the resultant extracts were transfered to glass dishes and were left in the incubator at 40ºC for 24 hrs. or until they dry, then they were left at 4ºC until assessment for their antimicrobial activities.

Test organisms:

Two gram (+) cocci, Staphylococcus aureus and Enterococcus spp. (Streptococcus faecalis) and three gram (-) bacilli Escherichia coli , Pseudomonas aeruginosa and Acinetobacter spp. were tested. These species of bacteria were originally isolated from skin- swap taken from out-patients suffering from skin lesions attended the hospital of medical city out-clinic in Baghdad. These patients were diagnosed by physician as having various skin infections. The swap samples were processed as soon as received by the laboratory, usually within few hours, and the isolated bacteria were identified using the method described by Forbes et al.[15]. Then, the isolated bacteria were maintained in the test tube slants of Mueller-Hinton agar medium at 37ºC for 24 hrs. and then stocked at 4ºC .Subcultures were prepared from the stocks for bioassay.

Agar well diffusion bioassay:

For bioassay a bacterial suspension of approximately 1.0×10^6 cell/ml in sterile normal saline was prepared as described by Forbes [16]. An aliquot of 1.0 ml was uniformly seeded on the nutrient agar media (15 ml, 4 cm thickness) in petri dishes. Wells of 6 mm in diameter and about 4 cm apart were punctured in the culture media using cork borers (16) , concentrations of (25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of each of plant leaves or seeds methanol extracts were prepared in dimethyl sulfoxide (DMSO): methanol (1:1 v/v) solvent (DMSO solvent), or the same concentrations of the plant aqueous extracts were prepared in sterile distilled water (1:1 v/v), and approximately 0.1 ml of each of the plant extract concentration mentioned above were administered to fullness in each well . The plates were then incubated at 37ºC for 48 hrs. After incubation, bioactivity was determined by measuring the diameter of inhibition zone (DIZ) in millimeter. All samples were tested in triplicate. Controls containing sterile DMSO or sterile distilled water without plant extracts were also employed, although no antibacterial activity was noted in the solvent used for the test.

Statistical analysis: Data of diameter of inhibition zone (DIZ), were presented as means of three replicates and were analyzed using factorial arrangement in complete random design (CRD) with SAS version 9 software

package [17]. Least Significant Differences (LSD) analysis was used to compare means. Significant differences were defined at $P < 0.001$.

Results and Discussion:

The effects of different concentrations of aqueous and methanolic extracts of leaves and seeds of Zizyphus spina-christi L. on five different species of bacteria Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Acinetobacter spp. and Enterococcus spp. isolated from skin infection, were studied in comparison with the standard antibiotic Tetracycline effects on these microorganisms (Table-5).

Aqueous extracts of leaves showed remarkable inhibition (17.67 mm) at concentration of (200 mg/ml) on Staphylococcus aureus and less inhibition (8.33 mm) and (0.00 mm) at lower concentrations (50 and 100 mg/ml) (table 1), while Pseudomonas aeruginosa showed lower response (7.33 mm, 8.00 mm and 10.00 mm) to various concentrations of leaves extracts (50, 100, and 200 mg/ml) respectively, on the other hand E. coli. and Enterococcus spp. were showed resistant to various concentrations of aqueous leaf concentrations, while Acinetobacter spp .growth inhibited with concentration of 200 mg/ml .

Leaves methanol extracts of concentrations (50, 100 and 200 mg/ml.) showed inhibitory effects against Staphylococcus aureus by (7.33, 10.33 and 12.00 mm) respectively and only on Acinetobacter spp. (8.33 mm) and Enterococcus spp. (13.33 mm) with concentration of (200 mg/ml), while other bacteria show no response (Table-2) . No effects were observed for seed aqueous extracts of all concentrations on all the species of bacteria tested (Table-3) except the concentration (200 mg/ml) which produced inhibition zone of (8.00 mm) on culture of Staphylococcus aureus, these obviously may need more concentrations of seed extracts for inhibition or it might have no effects at all on these bacteria. On the other hand, methanolic seed extracts showed antibacterial activity at only higher concentrations (100 mg/ml) and (200 mg/ml) with respect to Staphylococcus aureus and (200 mg/ml) with respect to E. coli, Acinetobacter spp. and Enterococcus spp. (Table-4). These results may indicate that leaf extracts have more antibacterial activity than seed extracts, and this finding may confirm the fact that leaf contain some constituents such as alkaloids, saponin, glycosides and flavonoids where the antimicrobial principle are related [12,13].

Al-Saimary [18,19], studied the effects of Zizyphus leaf aqueous extracts on Staphylococcus aureus and found that (100 mg/ml) and (250 mg/ml) have no effects on the growth of Staphylococcus aureus, while higher concentrations (500 mg/ml) and (750 mg/ml) inhibit the growth by about (9.00 mm) and (12 .00 mm) respectively. In our study, much lower concentrations of leaf extracts (100 mg/ml) and (200 mg/ml) inhibited the growth of Staphylococcus aureus by (8.33 mm) and (17.67 mm) respectively. This high antibacterial efficiencies of leaves extracts might due to the better extraction efficiency or due to variation in the quality or compositions of the same plant species or could be due to differences

in the environmental conditions and genetic variations. However, Smith et al. [20] recall that the emergence of Staphylococcus aureus resistance strains threaten to return to the era before the development of antibiotics. On the other hand, E.coli, Acinetobacter spp. and Enterococcus spp. were found to be susceptible only to high concentrations (200 mg/ml) of seed methanolic extracts, and their inhibition zone were (8.00, 7.67 and 7.67 mm) respectively, whereas high concentration (200 mg/ml) of leaf methanolic extract was inhibited the growth of Acinetobacter spp. and Enterococcus spp. by (8.33 and 13.33 mm) respectively , and this might indicate that the methanol probably extract different antimicrobial agents from leaves and seeds of Zizyphus spina-christi plant. In comparison with standard antibiotic, (Table-5) shows inhibition of Tetracycline (30 mg) against the same bacterial species isolated from the skin infections. All the results presented in this paper were statistically within 99.99 % confidence limits. These results agree with the results of Mutamedi et al. [21] where they showed that ethanolic and methanolic extracts from the Zizyphus spina-christi has no effects on the growth of E. coli and Pseudomonas aeruginosa with the concentrations of (50, 100 and 200 mg/ml) , and also supported by the results of Nasif (16) and Ali et al. [22] . In contrast with the study of Ali- Shtayah et al. [23] where they found that ethanolic extract of Zizyphus spina-christi was active against E. coli and Pseudomonas aeroginosa and they relate this antimicrobial activity to the unsaturated fatty acids which represent the major components (83.5 %) of ethanolic extract.

From the preceding results, it is obvious that, if the concentrations of various leaves and seeds extracts were increased above the concentrations used against the studied microorganisms, it would be possible to get high inhibitory effects than what it has been obtained in this paper. The problem of antibiotic resistance in both hospital- acquired (nosocomial) and community- acquired bacterial infection have made many antibiotics virtually obsolete, and also , it is well known that no antibiotics can last effective too long. Therefore, we recommend the use of all the parts of Zizyphus spina-christi plants (leaves, seeds, fruits ,barks and root bark) and at the same time searching through Iraqi flora extensively to investigate the bioactive compounds for use as therapeutic agents for treatment of inflammatory and infectious diseases. And this effort would support confidently the fact that the herbal remedies play a fundamental role in traditional medicine.

Bacterial Species	Inhibition Zone (mm)		
	Conc. mg		
	50	100	200
Staphylococcus aureus	0	8.33*	17.67*
Pseudomonas aeruginosa	7.33	8.00*	10.00*
E. coli	0	0	0
Acinetobacter spp	0	0	7.67
Enterococcus spp	0	0	0

Table-1: The effect of leaf aqueous extract on the growth of bacteria isolated from the skin.
LSD = 2.070 *Significant at P < 0.001

Bacterial Species	Inhibition Zone (mm)		
	Conc. mg		
	50	100	200
Staphylococcus aureus	7.33	10.33	12.00*
Pseudomonas aeruginosa	0	0	0
E. coli	0	0	0
Acinetobacter spp	0	0	8.33
Enterococcus spp	0	0	13.33*

Table-2: The effect of leaf methanolic extract on the growth of bacteria Isolated from the skin.
LSD = 1.180 *Significant at p < 0.001

Bacterial Species	Inhibition Zone (mm)		
	Conc. mg		
	50	100	200
Staphylococcus aureus	0	0	8.00*
Pseudomonas aeruginosa	0	0	0
E. coli	0	0	0
Acinetobacter spp	0	0	0
Enterococcus spp	0	0	0

Table-3: The effects of seed aqueous extracts on the growth of bacteria isolated from the skin.
LSD = 0.4794 *Significant at P < 0.001

Bacterial Species	Inhibition Zone (mm)		
	Conc. mg		
	50	100	200
Staphylococcus aureus	0	8.33*	10.00*
Pseudomonas aeruginosa	0	0	0
E. coli	0	0	8.00*
Acinetobacter spp	0	0	7.67*
Enterococcus spp	0	0	7.67*

Table 4: The effects of seed Methanolic extracts on the growth of bacteria isolated from the skin.
LSD = 0.8886 *Significant at 0.001

Bacterial Species	Inhibition Zone (mm)
Staphylococcus aureus	22
Pseudomonas aeruginosa	24
E. coli	20
Acinetobacter spp	16
Enterococcus spp	12

Table-5: The effect of Tetracyclin (30 mg) on the growth of bacteria isolated from the skin.
The inhibition zone represent mean of three replicates.

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