Evaluation of antimicrobial activity of aqueous extract of *Prosopis farcta* pods

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Abstract

The present study has been designated to evaluate the antimicrobial activity of aqueous extract of *Prosopis farcta* pods. The antimicrobial activity of the extract against five fungal strains and four bacterial strains were tested by using Agar Well Diffusion method and minimal inhibitory concentration MIC values. The results showed that aqueous extract of *P.farcta* had antifungal and antibacterial effects against all the tested microorganisms, wherease Nystatin failed to show any effect against *C.cladosporides*. Therefore, these results suggest that the aqueous extract of *P. farcta* possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of microbial diseases in human.

تقدير الفعالية المضادة للمايكروبات للمستخلص المائي لثمار الخرنوب *Prosopis farcta*

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المستخلص

صممت الدراسة الحالية لاختبار فعالية المستخلص المائي لثمار نبات الخرنوب *Prosopis farcta* على خمسة سلالات فطرية وأربعة سلالات بكتيرية وذلك باستخدام طريقة الحفر Agar Well Diffusion اضافة الى تحديد التراكيز المثبطة الدنيا MIC من المستخلص لكل نوع مايكروبي. اظهرت النتائج بان المستخلص المائي لنبات الخرنوب *P. farcta* يمتلك فعالية مضادة لجميع الفطريات والبكتريا المستخدمة في الدراسة، بينما فشل عقار Nystatin في اظهار أي تاثير ضد *C.cladosporides*. لذلك ومن خلال هذه النتائج يمكن القول ان المستخلص المائي للخرنوب *P. farcta* يمتلك مركبات ذات خصائص مضادة للمايكروبات والتي يمكن استخدامها في الادوية المستقبلية لمعالجة الامراض المايكروبية التي تصيب الانسان.

Introduction

Prosopis farcta(B.&S.)Eig.,is a member of the Liguminosae family,it is widely distributed all over Iraq except mountain areas in the north and locally named as 'Kharnub' [1]. The pods of *P.farcta* has been used traditionally as antidysentric and antispasmodic agents, also, it has showed antibacterial and antifungal properties [2,3,4,5].In recent years, many drug resistance pathogenic microorganisms have been appeared because of indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases [6], this leads to growing interest in looking for alternatives for these drugs from various sources, specially,medicinal plants.The present study was conducted to investigate antimicrobial properties of aqueous extract of *P.farcta.*

Materials and Methods

Plant material

Dry pods of *P. farcta* were collected from campus of Tikrit University, where they are growing wild widely. Plant material were authenticated and deposited at Science Faculty Herbarium, Tikrit University (No.5628).

Preparation of extract

Aqueous extract of *P. farcta* was prepared using the method described by Abdel-Barry *etal.* [7]. Briefly, dried pods squashed and the seeds were removed. The remain parts were grinded by electric grinder(Glasscow, India)to a fine powder. Fifty grams of the powder were suspended in 250ml of distilled water and then stirred magnetically (GallenKamp, England) for 24 hours at 50 °C. Subsequently, the suspension was filtered(Whatman No.1)and concenterated under reduced pressure (Rotary evaporator; Yamato, Japan) at 40°C,to get dry residue 6.55g (13.1%).

Microbial strains

A total 9 microbial cultures belonging to 5 fungi and 4 bacteria species,were used in this study.All the fungal and bacterial strains were isolated from clinical cases, fungal strains are: *Candida albicans,Cladosporium cladosporides,Cryptococcus neoformans,Tricho-phyton mentagrophytes,Trichophyton violacium* and bacterial strains are: *Escherichia coli Pseudomonas aeruginosa, Staphylococcus aureas, Streptococcus pyogenes*.Standard cultural and biochemical tests were used to identified all funagal and bacterial strains [8,9].

Antimicrobial activity

In fungal strains

Preparation of fungi inoculum

Fungal inoculum was prepared using the method described by MeGinnis [10], which is as follow:

1- Normal saline solution was prepared by dissolving 0.89g of Nacl in 100ml distilled water and divided into test tubes(5ml in each), sterilized in autoclave at 121°C and 15 Ib/Inch2 for 15min., and left to cool to 25°C.

2- The fungal strains were reactivated by growing them on Sabouroud Dextrose Agar(SDA)medium at 25°C. Fungal growth of 2-5 days old for yeast and of 2 weeks old for dermatophyes were taken by loop and transfered to test tubes containing sterile normal saline and shaked for short time.

3- Fungal inoculum of 10^6 conidia/ml was prepared using haemocytometer and measuring the optical density using a spectrophotometer(Cecil, England)at 540nm.

4- Test tubes were labelled and stored in cool place at 4°C until use.

Assay of plant extract concentrations

Agar Well Diffusion method was used [11], by pouring 20ml of SDA in a petridish (9cm diameter). The medium was inoculated with 0.1ml of 10^6 conidia/ml by sperading, the plates were left for 30min., then four wells (8mm diameter)were done by cork porer. 100ml of plant extract was added to each well by micropipette. The plates

were incubated at 25°C, then results were read after 2-5 days of incubation through measuring the diameter of inhibition zone. Different dilutions of the extract were used. The extract and standard antifungal agent was dissolved in dimethyl sulfoxide(DMSO)100% (biologically inert substance, which also used as negative control).

MIC assay

Minimal inhibitory concentrations (MIC)were determined as described by Nostro *etal.* [12] through mixing 2ml of each concentration (2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003 mg/ml) with 18ml of cooled SDA medium. Then pouring in petridishes, one of them served as control (without extract), 0.1ml of the inoculum 10^6 condi/ml was cultured as small spot on SDA medium as mentioned previously. The plates were incubated at 25°C and the results were recorded.

In bacterial strains

Assay of plant extract concentration

The Agar Well Diffusion method were used by pouring 20ml of Muellar-Hinton Agar(MHA)for each petridish. The medium was inoculated with 0.1ml of 0.1optical density of bacterial suspension. The procedure is the same that mentioned previously,except that Gentamicin(10 mg/ml)was used as positive control.

MIC assay

Two milliliter of each plant extract was used (2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003 mg/ml) with 18ml of MHA. The mixture poured in petridish to obtain the final concentration, one of the petridishes which was without extract served as control. 0.1ml of bacterial inoculum was cultured as small spots on MHA medium and plant extract mixture. The plates incubated at 37°C for 24h. and the results were recorded [13].

Statistical analysis

The values are given as mean±SD . The data was analyzed by ANOVA followed by post hoc test using computerized software SPSS(Version 7.5). *P*-value less than 0.05 were defined as statistically significant.

Results

The results showed that the effect of *P.farcta* was dependent on the concentration. The *P. farcta* concentrations(0.25, 0.5 mg/ml)in *C.albicans*,(0.25mg/ml)in *C neoformans*,(4mg/ml)in *T. mentagrophytes* and(1 mg/ml)in *T. violacium*, was similar in their antifungal effect to the positive reference standard(Nystatin),while inhibition zones in higher concentrations of extract was significantly higher than Nystatin in their effect against the fungal strains. Nystatin showed no effect against *C. cladosporides* and its effect was better than(0.25-2 mg/ml)in*T. mentagrophytes* and(0.25, 0.5 mg/ml) in *T. violacium* of *P. farcta* (Table 1).The results of table 2 shows that the concentrations of *P. farcta*(4-128 mg/ml) in *E. coli*, (1-128 mg/ml) in *P. aeruginosa*,(2-128 mg/ml)in *S. aureas* and (8-128 mg/ml) in *S. pyogenes*, have significantly higher effect than Gentamicin. While the other concentrations of P. farcta was similar or less activity than Gentamicin against the tested bacterial strains.In the tested fungal strains, *C.neoformans* and *T. violacium* have the lowest recorded MIC(0.7µg/ml),while the highest MIC is recorded against*Ccladosporides* and *T. mentagrophytes* (6.2 µg/ml) (Table 3).

Table 4 shows the MIC of *P. farcta* against the bacterial strains, the lowest recorded MIC (1.5 µg/ml) was for *E. coli*, *P.aeruginosa* and *S. aureas*, the highest MIC is recorded against *S. pyogenes* (25 µg/ml).

Table (1) Antifungal activity of *P.farcta* based on Agar well diffusion method

Concentration mg/ml	Inhibition zone diameters (mm) (mean ± SD)				
	Candida albicans	*Cladosporium cladosporides*	*Cryptococcus neoformans*	*Trichophyton mentigrophytes*	*Trichophyton violacium*
128	17.0 ± 0.81 a	10.6 ± 0.64 a	12.0 ± 0.29 a	11.6 ± 0.17 a	11.6 ± 0.38 a
64	16.0 ± 0.37 b	10.3 ± 0.16 ab	11.6 ± 0.63 ab	11.3 ± 0.37 ab	11.3 ± 0.21 b
32	15.0 ± 0.16 c	10.0 ± 0.29 bc	11.3 ± 0.17 bc	11.0 ± 0.16 bc	11.0 ± 0.21 c
16	14.6 ± 0.35 cd	9.6 ± 0.31 cd	11.0 ± 0.62 cd	10.6 ± 0.71 c	10.6 ± 0.86 d
8	14.3 ± 0.28 de	9.3 ± 0.29 de	10.6 ± 0.16 de	10.0 ± 0.53 d	10.3 ± 0.25 e
4	14.0 ± 0.14 ef	9.0 ± 0.84 ef	10.3 ± 0.33 ef	9.6 ± 0.11 de	10.0 ± 0.16 f
2	13.6 ± 0.12 fg	8.6 ± 0.21 fg	10.0 ± 0.16 fg	9.3 ± 0.21 ef	9.6 ± 0.38 g
1	13.3 ± 0.21 g	8.3 ± 0.52 gh	9.6 ± 0.57 gh	9.0 ± 0.62 fg	9.3 ± 0.14 h
0.5	12.0 ± 0.42 h	8.0 ± 0.21 h	9.3 ± 0.24 hi	8.6 ± 0.29 gh	9.0 ± 0.16 i
0.25	11.6 ± 0.17 h	7.0 ± 0.48 i	9.0 ± 0.21 i	8.3 ± 0.11 h	8.3 ± 0.21 j
Nystatin 0.25 mg/ml	12.0 ± 0.92 h	0.0 ± 0.0 k	9.0 ± 0.78 i	9.6 ± 0.43 de	9.3 ± 0.19 h
Negative control DMSO 100%	0.0 ± 0.0 k				

Vertically: the different letters means there are statistically significant difference.

Table (2) Antibacterial activity of *P.farcta* based on Agar well diffusion method

Concentration mg/ml	Inhibition zone diameters (mm) (mean ± SD)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureas*	*Streptococcus pyogenes*
128	11.0 ± 0.24 a	12.0 ± 0.56 a	13.0 ± 0.21 a	9.6 ± 0.14 a
64	10.6 ± 0.14 b	11.6 ± 0.37 b	12.6 ± 0.14 b	9.3 ± 0.22 b
32	10.3 ± 0.16 bc	11.3 ± 0.84 bc	12.3 ± 0.17 bc	9.0 ± 0.16 c
16	10.0 ± 0.84 c	11.0 ± 0.11 c	12.0 ± 0.16 c	8.6 ± 0.11 d
8	9.6 ± 0.12 d	10.6 ± 0.24 d	11.0± 0.70 d	8.3 ± 0.74 e
4	9.3 ± 0.21 de	10.3 ± 0.19 de	10.6 ± 0.32 e	8.0 ± 0.23 f
2	9.0 ± 0.15 e	10.0 ± 0.14 e	10.3 ± 0.26 ef	7.6 ± 0.68 g
1	8.6 ± 0.63 f	9.6 ± 0.38 f	10.0 ± 0.18 f	7.3 ± 0.14 h
0.5	8.3 ± 0.61 fg	9.3 ± 0.14 fg	9.6 ± 0.14 g	7.0 ± 0.16 i
0.25	8.0 ± 0.23 g	9.0 ± 0.29 g	9.3 ± 0.96 g	6.6 ± 0.84 j
Gentamicin 10 mg/ml	9.0 ± 0.18 e	9.3 ± 0.49 fg	10.0 ± 0.28 f	8.0 ± 0.47 f
Negative control DMSO 100%	0.0 ± 0.0 k			

Vertically: the different letters means there are statistically significant difference.

Table (3) MIC values of *P.farcta* against fungal strains

Fungal species	MIC (µg/ml) for aqueous extract of *P.farcta*
Candida albicans	1.5
Cladosporium cladosporides	6.2
Cryptococcus neoformans	0.7
Trichophyton mentigrophytes	6.2
Trichophyton violacium	0.7

Table (4) MIC values of *P.farcta* against bacterial strains

Bacterial species	MIC (µg/ml) for aqueous extract of *P.farcta*
Escherichia coli	1.5
Pseudomonas aeruginosa	1.5
Staphylococcus aureas	1.5
Streptococcus pyogenes	25

Discussion

The antimicrobial activity of *P. farcta* aqueous extract was tested in the present study and its potency was assessed by the presence or absence of inhibition zones, zone diameters and MIC values.*P. farcta* was active against all tested fungal strains(*Candida albicans, Cladosporium cladosporides*, *Cryptococcus neoformans, Trichophyton mentigrophytes* and *Trichophyton violacium*), also the extract was active against Gram +ve (*Staphylococcus aureas* and *Streptococcus pyogenes*)and Gram –ve (*Escherichia coli* and *Pseudomonas aeruginosa*)bacteria, which is agree with Sokmen *etal* [14], and disagree with other studies that showed Gram +ve bacteria more sensitive to plant extracts than Gram –ve bacteria [15,16] or vice-versa [17].

The extract may be work by one or both of the following mechanisms: disrup cytoplasmic membrane of the microorganisms by its effect on lipids and proteins [18] or penetration the active sites of specific enzymes inside the microorganisms which is imported for their multiplication [19].The basis of varying degree of sensitivity of tested microorganisms both of bacteria and fungi to plant extract may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the plant extract. Alkaloids, tannins and glycosides were the major phytochemicals that detected in *P.farcta* [3,20], these phytochemicals are known to have antimicrobial activity [21].Further studies of *P.farcta* and

isolated compounds are necessary to develop a potent antimicrobial drugs.

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