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Antimicrobial activity of aqueous extract of Citrullus colocynthis L.fruit

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Abstract

The aqueous extract of C. Colocynthis fruit was tested for its antimicrobial activity against certain pathogenic fungal and bacterial isolates using agar well diffusion method. The activity of the extract was determined through measuring the diameter of inhibition zone and minimal inhibitory concentration. The study revealed that all the fungal and bacterial isolates are sensitive to the extract . Fungal isolates are more sensitive than bacterial isolates. All concentrations of the extract used in this experiment showed antifungal activity against the Cladosporium cladosporides.

The activity of Citrullus colocynthis L. extract is significantly better than the standard antifungal drugs, nystatin and fluconazole in a concentration of 0.25 mg/ml .The inhibitory effect of C. colocynthis fruit aqueous extract may attributed to active compounds present in the extract.

الفعالية المضادة للمايكروبات للمستخلص المائي لثمار الحنظل

Citrullus colocynthis L.

محمد لطيف الكامل

المستخلص

أجريت الدراسة الحالية لاختبار فعالية المستخلص المائي لثمار الحنظل Citrullus colocynthis ضد عدة عزلات من الفطريات والبكتريا المرضية وذلك باستخدام طريقة الحفرAgar well diffusion وقدرت فعالية المستخلص من خلال قياس قطر منطقة التثبيط Inhibition zone وتحديد التراكيز المثبطة الدنيا (MIC) من المستخلص لكل نوع من العزلات المستخلصة. اضهرت النتائج فعالية المستخلص ضد جميع الفطريات والبكتريا المستخدمة وكانت حساسية العزلات الفطرية تجاه المستخلص افضل من العزلات البكتيرية . وكانت الخلاصة المائية لثمار الحنظل ولجميع التراكيز المستخدمة قد اعطت تاثيرا مضادا للفطرCladosporium cladospordes أفضل من تاثير المضاد الفطري النستاتين والفلوكونازول بتركيز 0.25 ملغم/مل . ظهر بان نبات الحنظل يحتوي على اكثر من مادة فعالة وان بعض هذه المواد لها فعالية مضادة للفطريات والبكتريا تفوق فعالية المضادات الفطرية والحياتية المعروفة .

Introduction

The plant *Citrullus colocynthis* L. scard is perennial herb belong to the family cucurbitaceae with branched tendrils . Very scabrid grey, with long trailing branches . Stem is angular very much branched, covered with adepressed coarse hairs. Leaves are narrowly triangular, 5-12 cm long deeply 3-7 lobed , with rounded sinuses. Flowers are small greenish-yellow. Receptacle is broadly campanulate cover with white , hispid hairs. Calyx with subulate sepals , recurved at the apex . Corolla with acute and mucuronate lobes. Young fruits are fleshy , globular , mottled with dark-green , turning dry and yellow when ripe, 10cm in diameter . Fruits are extremely bitter. Seeds are smooth and shining [1] .

Citrullus colocynthis is distributed in desert areas **in** Iraq during the period from May to October.The fruits of *Citrullus colocynthis* contain many active compounds including colocynthidin and colocythin alkaliods, bitter resins, saponin and glycosides like cucurbitacin E [2 , 4 , 5] In traditional medicine , *Citrullus colocynthis* fruits were used for the treatment of Jaundice, constripation , fever, treatment of intestinal parasite and Amenorrhea[3].A lot of efforts were spend to investigate an alternative therapies of plant source because of development of many resistance microbial strains and awide range of side effects appeared with chemotherapy.

The purpose of this study is to investigate the possible antimicrobial activity of aqueous extract of colocynth fruits against some fungi and bacteria.

Materials and Methods

Collection and Identification of plant samples :

Riped fruits of colocynth were collected from area near Tikrit university , Then they were sended to be identified by the Iraq National Herbarium .The fruits were dried in shadow , squashed and the seeds were removed . The remains were grind by electric grinder and the powder were kept in a plastic bag.

Extraction of plant sample:

The method mentioned by [6] was followed for extraction of plant sample.

Fifty grams of fruit powder of colocynth were mixed with 250ml of distilled water and then stirred by magnetic stirrer for 24hrs at 50C° .The suspension was filtered by Whatman (No.l) filter paper , then the extract was dried by rotary evaporator and kept in glass vials at 20 C° until used.Fungal and bacterial isolates:

Five fungal and four bacterial isolates were used to test the antimicrobial activity of colocynth fruit . All the fungal and bacterial isolates used in this study were isolated from clinical cases which include *Trichophyton mentagrophytes* , *T. Violacium* , *Cladosporium cladosporides* , *Candida albicans* and *Cryptococcus neoformans*., *Staphylococcus aureas* , *Streptococcus pyogenes* , *Pseudomonas aeruginosa* and *Escherichia coli*. Standered cultural and biochemical tests were used to identified all fungal and bacterial isolates [7-10]

In vitro testing of the biological activity of the aqueous extract:

Study of the effect of aqueous extract on growth of fungi: Preparation of fungal inoculum:

Fungal inoculum were prepared followed the method of MeGinnis[8].

1.Normal saline solution was prepared by dissolving 0.89gm of NaCl in 100ml distilled water and

distributed in test tubes (5ml in each) , sterilized in autoclave at 121 C° and 15(lb/Inch^2) for 15 min., and left to cool to 25 C°.

2.The fungal isolates were reactivated by growing them on SDA medium at 25C° . Fungal growth of 2-5 days old for yeasts and of 2 weeks old for dermatophytes 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 540 nanometer .

4.Test tubes were labelled and stored in acool place at 4 C° until use.Preparation of agar plates to test the different concentrations of plant extract:

Agar well diffusion method was used [11] by pouring 20ml of Sabouroud Dextrose Agar SDA in a petridish (9 cm diameter) .The medium was inoculated with O.lml of 10^6 conidia/ml by sperading , the plates were left for 30 minutes , then four wells (8 mm diameter) were done by cork porer. 100 µl of plant extract was added to each well by micropipette .The plates were incubated at 25 C°. The results were read after 2-5days of incubation by measuring the diameter of inhibition zone .Different dilutions of the extract were used. The extract and standerd antifungals were dissolved in dimethyl sulfoxide (DMSO) 100% (biologically inert substance ,which also used as negative control).

Determination the minimal inhibitory concentrations (MIC) for the plant extract on the growth of fungi:

Minimal inhibitory concentration were determined following the method of [12] by 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 petridish with out extract was used to represent control, 0.1 ml 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.

-Study of the effect of C. *colocynthis* fruit extract on the growth of bacteria:

The agar well diffusion method were used by pouring 20ml of Muellar-Hinton Agar(MHA) for each petridish .The medium was inoculated with 0.lml of 0.1 optical density of bacterial suspension .The procedure is the same that mentioned before. except that gentamicin (10µg/disc , Oxoid) was used as positive control.

Determination of minimal inhibitory concentration of plant extract on bacterial growth:

2ml of each plant extract was used (2 , 1 , 0.5 , 0.25 , 0.125 , 0.062 , 0.031 , 0.015 , 0.007 , 0.003 mg/ml) with 18ml ofMHA. The mixture poured in petridish to obtain the final concentration. One petridish without extract added to represent control. Bacterial inoculum was prepared, O.lml of the inoculum was cultured as small spot on MHA medium mixing with plant extract .The plates incubated at 37C° for 24 hrs and the results were recorded [13].

Results

The effect of C. *Colocynthis* fruit extract on the growth of fungi:

The results revealed that the effect of the aqueous extract of C. *colocynthis* fruit on 5 fungal species was depend on the concentration of the extract and the fungal species. Table 1 showed that the effect of extract in a concentration of 0.5-32 mg/ml on *Trichophyton mentagrophytes* is similar to that of nystatin and fluconazole in a concentration of

0.25mg/ml .While its effect in a concentration of 64-128mg/ml is significantly better than nystatin in a concentration of 0.25 mg/ml ($P<0.01$).

The effect of *C. colocynthis* extract in a concentration of 1-128 mg/ml on *T. violaceum* is similar to that of the two standred antifungals in a concentration of 0.25mg/ml. The effect of extract in all concentrations on *Cladosporium cladosporides* is significantly better than nystatin and fluconazole in a conentration of 0.25 mg/ml ($P<0.001$). The effect of extract in a concentration of 0.25-4 mg/ml is similar to that of nystatin and fluconazole in a concentration of 0.25mg/ml.

However, the extract in a concentration of 8-128mg/ml exerts effects significantly more than that exerted by the two standared antifungals in a concentration of 0.25mg/ml ($P<0.01$) a gainst *Candida albicans* .The effect of extract in a concentration of 0.25-8mg/ml against *Cryptococcus neoformans* is similar to that of nystatin and fluconazole in a concentration of 0.25mg/ml , while it's effect in a concentration of 16-128mg/ml is significantly better than that of nystatin in a concentration of 0.25mg/ml ($P<0.01$).

Table 1 showed that the effect of all concentrations of the aqueous extract of C. *Colocynthis* is significantly better than the effect of negative control, dimethyl sulfoxide ($P<0.0001$) against all species of examined fungi.The effect of extract of colocynth fruits against the growth of 4 species of pathogenic bacteria:

It appeared that the effect of aqueous extract was depend on it's concentration and the species of the studied bacteria. The mean of diameter of the inhibition zone is positivity correlated to the concentration of the extract. Table 2 show that effect of the extract in a concentration 0.25-16mg/ml is similar to that of gentamycin in a concentration of 10µg/disc against *Staphylococcus aureas* while the effect of extract in a concentration of 0.25-64mg/ml exerted same effect exerted by gentamycin in a concentration of 10µg/disc against *Streptococcus pyogenes* .However , the effect of extract in a concentration of 0.25-64mg/ml is similar to that of gentamycin in a concentration of 10µg/disc against *Pseudomonas aeruginosa,* while the effect of the extract in a concentration of 0.25-32mg/ml against *E.coli* is similar to that of gentamycin in a concentration of 10µg/disc , However when the concentration increased to 128mg/ml , it's effect became significantly better than gentamycin ($P<0.01$). On the other hand , the effects of all concentration of the aqueous extract of colocynth are better than negative control ($P<.0001$) against all speices of examined bacteria.

The minimal inhibitary concentration (MIC) for the aqueous extract of C. *colocynthis* against the growth of fungal and bacterial isolates: Table 3 showed that the (MIC) of the extract against the growth of 5 species of pathogenic fungi.The least (MIC) is that recorded against *Candida albicans & Cryptococcus neoformans* , which is 1.5µg/ml , while the highest (MIC) is that recorded against *Cladosporium cladosporides* which is 6.2µg/ml Table 4 showed the (MIC)of the extract against the growth of 4 species of pathogenic bacteria .The least (MIC) is that recorded against *S. aureas & E. coli* which is 1.5µg/ml , while the higest (MIC) is that recorded against *S. Pyogenes* which is 6.2µg/ml.

Table (1): The effect of aqueous extract of *C. colocynthis* fruits against the growth of fungal isolates

Concentration mg / ml	Inhibitory zone diameters (mm) (mean ± SD)				
	Trichophyton mentagrophytes	*Trichophyton violacium*	*Cladosporium cladosporides*	*Candida albicans*	*Cryptococcus neoformans*
128	13.0 ± 0.5 a	12.0 ± 0.5 a	8.60 ± 0.5 a	17.0 ± 0.5 a	14.0 ± 0.5 a
64	12.6 ± 1.0 a	11.6 ± 0.5 a	8.30 ± 1.0 a	15.0 ± 0.5 ab	13.0 ± 0.5 a
32	12.3 ± 0.5 ab	11.3 ± 1.0 ab	8.00 ± 0.5 a	14.0 ± 1.0 abc	12.6 ± 0.5 a
16	12.0 ± 0.5 ab	11.0 ± 0.5 ab	7.60 ± 0.5 a	14.0 ± 0.5 bc	12.0 ± 0.1 a
8	11.6 ± 0.5 ab	10.6 ± 1.0 ab	7.30 ± 1.0 a	13.0 ± 0.5 bc	11.6 ± 0.5 ab
4	11.3 ± 0.5 ab	10.3 ± 0.5 ab	7.00 ± 0.5 a	12.0 ± 0.5 cd	11.3 ± 0.5 ab
2	10.6 ± 0.5 abc	9.60 ± 0.5 ab	6.60 ± 1.0 a	11.6 ± 0.5 cd	11.0 ± 1.0 ab
1	10.0 ± 0.5 bc	9.30± 0.5 ab	6.40 ± 0.5 a	11.0 ± 1.0 cd	10.6 ± 0.5 b
0.5	9.00 ± 1.0 bc	9.00 ± 0.5 b	6.10 ± 0.5 a	10.0 ± 1.0 cd	10.0 ± 0.5 b
0.25	8.00 ± 0.5 c	8.60 ± 1.0 b	6.00 ± 0.5 a	9.00 ± 0.5 d	9.00 ± 1.0 b
Nystatin 0.25 mg/ml	9.60 ± 0.5 bc	9.30 ± 1.0 ab	6.00 ± 0.5 a	12.0 ± 0.5 d	9.00 ± 0.5 b
Fluconazole0.25 mg/ml	12.0 ± 0.5 ab	12.0 ± 0.5 a	0.00 ± 0.0 b	10.0 ± 1.0 d	11.6 ± 0.5 ab
Negative control DMSO 100%	0.0 ± 0.0 e				

Vertical: The same letters mean there is no statistically significant difference.

Table(2) : The effect of aqueous extract of *C. colocynthis* fruits against the growth of bacterial isolates

Concentration mg / ml	Inhibitory zone diameters (mm) (mean ± SD)			
	Staphylococcus aureas	*Streptococcus pyogenes*	*Pseudomonas aeruginosa*	*Echerichia coli*
128	12.0 ± 0.5 a	11.0 ± 0.5 a	11.0 ± 0.5 a	12.0 ± 0.5 a
64	11.6 ± 0.5 ab	10.6 ± 1.0 ab	10.3 ± 0.5 ab	11.0 ± 1.0 ab
32	11.3 ± 1.0 ab	10.3 ± 0.5 ab	10.0 ± 1.0 ab	10.6 ± 0.5 abc
16	10.0 ± 0.5 abc	10.0 ± 0.5 ab	9.60 ± 0.5 ab	9.60 ± 0.5 abc
8	9.60 ± 0.5 abc	9.60 ± 1.0 ab	9.30 ± 0.5 ab	9.30 ± 0.5 abc
4	9.30 ± 0.5 abc	9.30 ± 0.5 ab	9.00 ± 0.5 ab	9.00 ± 1.0 bc
2	9.00 ± 1.0 bc	9.00 ± 1.0 ab	8.60 ± 0.5 ab	8.60 ± 1.0 bc
1	8.60 ± 0.5 bc	8.60± 0.5 b	8.30 ± 1.0 ab	8.30 ± 0.5 bc
0.5	8.30 ± 0.5 c	8.00 ± 0.5 b	8.00± 0.5 b	8.00 ± 1.0 c
0.25	8.00 ± 1.0 c	7.00 ± 0.5 b	7.00 ± 0.5 b	7.60 ± 0.5 c
Gentamycin 10 µg / disc	10.00 ± 0.5 abc	8.00 ± 0.1 b	9.30 ± 1.0 ab	9.00 ± 0.5 bc
Negative control DMSO 100%	0.0±0.0 d			

Vertical: The same letters mean there is no statistical significant difference.

Table (3) : MIC of the aqueous extract of *C. colocynthis* against fungal isolates

Fungal speices	MIC (µg / ml) for aqueous extract of *C. colocynthis* fruit
Trichophyton mentagrophytes	3.1
Trichophyton violacium	3.1
Cladosporium cladosporides	6.2
Candida albicans	1.5
Cryptococcus neoforamans	1.5

Table (4) : MIC of the aqueous extract of *C. colocynthis* against bactetial isolates

Bacterial speices	MIC (µg / ml) for aqueous extract of *C. colocynthis* fruit
Staphylococcus aureas	1.5
Streptococcus pyogenes	6.2
Pseudomonas aeruginosa	3.1
Echerichia coli	1.5

Discussion

The present study revealed that fungal isolates are more sensitive than bacterial isolates toward the aqueous extract of C. *colocynthis* fruits based on the effective concentration .The same results were recorded by Aqil and Ahmed [14]. The inhibitory action of the extract could be attributed to the presence of active compounds in the extract which are water soluble like glucosides and resins which inhibit enzymatic activity in cytoplasmic membrane [15].

The extract inhibited the growth of all tested fungal isolates , this attributed to the presence of the active compounds, colocynthidin and colocynthin alkaloids which may be disrupt cytoplasmic membrane of the microorganisms through their action on lipids and protein [16], furthermore these compounds may penetrated cytoplasmic membrane and competed the active sites of certain enzymes inside the cell that are essential for multiplication of the microrganisims[17] .The extract exerted inhibitory effect on both gram-positive and gram-negative bacteria ,the present result agree with the study of [18] which showed that it kills bacteria in respective to their cell wall structure ,however other studies showed that gram-positive bacteria is more sensitive than gram-negative bacteria [19,20] or vice-versa[21] .

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