الفعالية المضادة الأحياء المجهرية للمستخلص المائي لخلاصة نبات الزنجبيل

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الملخص

التأثير المايكروبي للمستخلص المائي لنبات الزنجبيل .اختبـرت فعاليتـة ضـد الفطريـات المبيضات البيض ، المبغثرة ، الشعروية البنفسجية، والمكورات الخبيئة **، مع أربع مـن البكتريـة وهي الإشريكية القولونية و الزائفة الزنجارية و المكورات العنقودية الذهبية و المكورات العقديـة المفيحة. يستخدم الزنجبيل لعلاج القيء في الطب الشعبي واختبرت فعاليتـه فـي المختبـر باستخدام طريقة الانتشار في الاكار والتركيز المثبط الأدنى بواسطة طريقة تخفيف الاجار إذْ من تتراوح من 1 إلى 5 إلى12 مايكرو غراماً وأكثر أنـواع 1 الفطريـات حساسـية هـي المبيضات البيض والتركيز المثبط الأدنى هو 1.5مايكرو غراماً والبكتريا القولونية والتركيز المثبط الأدنى هو 1.5 مايكرو غراماً.**

مفاتيح الكلمات: التأثير المايكروبى والزنجبيل

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Antimicrobial Activity of Aqueous Zingiber Officinalis Root

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Abstract

The antimicrobial activity of aqueous extract of Zingiber Officinalis were tested for their antifungal activity against the following dermatophytes :Candida albicans, Cladosporium cladosporiosis , Cryptococcus neuphormans, Trichophyton violaceum and against other four bacteria : E. coli, Pseudomonas aeruginosa. Staphylococcus aureus, Streptococcus pyogenes. The extract used in traditional medicine for the treatment of nausea was tested in vitro through the Agar Disk Diffusion Method. The minimum inhibitory concentration (MIC) of extracts determined by the Agar dilution method ranged from 1.5 to 12.5 mcg. The most sensitive microorganisms to the extract were Candida albicans with MIC 1.5 and E coli with MIC 1.5 mcg.

Key words: Antimicrobial, Zingiber Officinalis

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Introduction

Herbal medicine has a long history in the treatment of several kinds of diseases (1). This development has also led to focusing research activities toward the chemical and microbiological loads of herbal medicinal products (2). The Arial parts of Zingiber Officinalis plant (Ginger)are used in traditional medicine in Iraq for the treatment of rheumatoid , arthritis, and stomach ulcer .(3,4,5,6). The aim of the present study was to evaluate antimicrobial activity of ginger plant against some bacterial and fungal pathogens.

Materials and methods

Plant material : Fresh Zingiber were purchased from a local market in Tikrit

Extraction Rhizomes of ginger were cut into small pieces and dried in the Tray – Dryer over at 600. They were pulverized separately in a hammer will and passed at 60-mesh sieve (7).

Fungal and bacterial isolates :

Five fungal and four bacterial isolates was tested, all the fungal isolates used in this study were isolated from clinical case which included *Candida albicans* ,*Cladosporium cladosporoids*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *T*. violaceum .The other four tested bacteria were *Escherichia coli* , *Pseudomonas aeruginosa* ,*Staphylococcus aureus* , and *Streptococcus pyogenes.*

Standard cultures and biochemical tests were used to identify all fungal and bacterial isolates(8,9,10).

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

Study of the effect of aqueous extract on growth of fungi;

1- Preparation of fungal inoculum:

Fungal inoculum were prepared following the method of **MEG innis** (9). Normal saline solution was prepared by dissolving 0.89 of NaCl in 100ml of distilled water and distributed in test tube (5ml in each one) then sterilized in autoclave at 121 c and 15 (lib/inch) for 15 min. then left to cool to 25 c

2- The fungal isolates were reactivated by growing them on SDA medium at 25 c. fungal growth of 2-5 days old for yeasts and 2 weeks old for dermatophytes were taken by loop and transferred to test tubes containing sterile normal saline and shacked for short time.

3-Fungal inoculum of 10 Candida /ml was prepared using haemocytometer and measuring the optical density was done by using a

spectrophotometer (10) at 540 manometer. Test tubes were labeled and stored in a cool place at 4 c until use

3- Preparation of agar plate to test the different concentrations of plant extract :Agar well diffusion method was used (11) by pouring 20 ml of SDA in a Petri dish (9 cm diameter). The medium was inoculated with 0.1 Candida /ml by spreading , the plates were left for 30 min then four wells (8mm diameter) done by cork paper , 100 ml of plant extract was added to each well by micropipette . The plates were incubated at 25 c. The results were read after 2-5 days of incubation by measuring the diameter of inhibition zone.

For studying antimicrobial activity of aqueous Zingiber officinalis root Different dilutions of extract were used. The extract and standard antifungal were dissolved in dimethyl sulfoxide (DMS) 100%(biologically inert substances and DMSO was also used as negative control).

Determinations of the minimal inhibitory concentrations were determined following the method of (12). By mixing 2 ml of each concentration (2,1,0.5, 0.25,0.125,0.062,0.031,0.015,0.007,0.003mg/ml) with 18 ml of cooled SDA media then pouring in Petri dish. One Petri dish with out extract was used to represent control. Fungal inoculum was prepared (10candida/ml)0.1 ml of the inoculum was cultured as small spot on SDA as mentioned previously . The plates were incubated at 25 c and the results were recorded.

Study of the effect of Zingiber officinal root extract on the growth of bacteria:

The agar well diffusion method were used by pouring 20 ml of Mueller Hinton agar for each Petri dish . The medium was inoculated with 0.1 ml of 0.1ml optical density of bacteria suspension. The procedure is the same that mentioned except that gentamycin (10m/disk,Oxoid was used as positive control .

Determination of minimal inhibitory concentration of plant extract on bacterial growth

2ml of each plant extract was used (2,1,0.5,0.125,0.062,0.031,0.015,0.007,0.003mg/ml) with 18 ml of MHA. The mixture poured in Petri dish to obtain the final concentration. One Petri dish with out extract added to represent control. Bacterial inoculum was prepared; 0.1ml of the inoculum was cultured as small spot on MHA medium mixing with plant extract.

The plates incubated at 37 c for 24 hrs and the results were recorded (12)

Results:

Table 1 summarize the colony growth of dermatophytes on subuarads agar with and without the plant extract and similar medium containing nystatin . As is shown ,the dried aqueous extract of Zingiber officinalis were effective in inhibiting the growth of the fungi . The effect of nystatin and isoconazole nature on *T.* mentagrophytes was less than that on the others four fungi i.e. *Candida albicans, Cladosporium. Cladosporides, Cryptococcus neophormans. T. violaceum.* It is clear from table 1 that the tested fungi varied in their effect .

Table 2 shows MIC of the aqueous extract of Zingiber officinal roots against fungal isolate .The MIC ranges from 1.5 to 3.1. Table 3 shows the effects of Zingiber officinalis root against the growth of bacterial isolates. Table 4 shows the MIC of the aqueous extract of Zingiber officinalis root against bacteria isolates ,the MIC ranges from 1.5 to 6.2.

Discussion:

Extract of Z. officinalis have been reported to posses numbers of biological activities (15,16) and have been used extensively in antinausea clinical trials (17,18) including trials conducted in pregnant women (19).

Dermatophytes have been studies in Iraq (20,21) and there are previous publication about the effects of some plants extract on such fungi and some bacteria (22,23). Our results support this direction as the presented results indicates that different plant aqueous extracts have different inhibitory effect on different species of fungi . For clinical purposes, we need to know exactly the causing fungal sepsis otherwise we should use a mixture of plant extract for the treatment.

This variation in antimicrobial effects also indicates presence of different effective compounds in the test plants, which could be extracted or purified from these plants (24). These results agree with Ficher (25,26) who reported that Ginger extract standardized on the basis of the identified compounds could be considered as antifungal agents in practical therapy . The results have encouraged us to undertake further studies regarding the isolation and characterization of the active compounds present in the active extract..

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Table1 the effect of aqueous extract of *Zingiber officinale* roots against the growth of fungal isolates

	Mean inhibitory diameter(mm)				
Concentration Mg/ml	*Candida albicans*	*Eladospoium cladosporoides*	*Cryptococcus neoformans*	*Trichophyton mentagraphytes*	*Trichophyton violacium*
128	16.0	8.3+-1.0	12.0+-1.0	13.0+-0.5	11.3+-0.5
64	14.3	8.0+-1.0	11.6+-1.0	12.6+-0.5	11.0+-1.1
32	13.0	7.6+-0.5	11.0+-0.5	12.0+-1.1	10.6+-0.5
16	12.3	7.3+-0.5	10.6+-0.5	11.6+-1.1	10.3 +-1.0
8	12.0	6.6+-1.1	10.3+-0.5	11.3+-0.5	10.0+-0.5
4	11.0	6.3+-0.5	10.0+-1.1	11.0+-0.5	9.6+-0.5
2	10.6	6.0+-1.0	9.6+-0.5	10.6+-1.0	9.0+-1.0
1	10.3	5.6+-0.5	9.3+-0.5	10.3+-1.0	8.0+-0.5
0.5	9.6	5.3+-1.0	9.0+-1.0	10.0+-0.5	7.6+-1.1
0.25	8.6	5.0+-1.0	8.6+-1.0	9.0+-0.5	7.3+-0.5
Isoconazole Nitrate 0.25mg/ml	10.0	14.0+-0.5	10.0+-1.0	15.0+-1.0	9.0+-0.5
Nystafin 0.25mg/ml	11.0	0.0+-0.0	9.0+-0.5	12.0+-0.5	9.3+-1.0
Negative Control DMSO 100%	0.0	0.0	0.0	0.0	0.0

Table 2 MIC of the aqueous extract of *Zingiber officinal* roots against fungal isolate

Fungal species	MIC(mg/ml)for aqueous Extract of Ziniber officinal roots
Candida albicans	1.5
Cladosporium cladosporides	12.5
Cryptococcus neophormans	6.2
Trichophyton mentagrophytes	6.2
Trichophyton violacium	3.1

Table 4 MIC of the aqueous extract of *Zingiber officinal* roots against bacterial isolates

Bacterial species	MIC(mg/ml)for aqueous Extract of Ziniber officinal roots
Echerichia coli	1.5
Pseudomonas aeruginosa	6.2
Staphylococcus aureus	3.1
Streptococcus pyogenes	6.2

Table 3 the effect of aqueous extract of *Zingiber officinal* roots against the growth of bacterial isolates

Concentration Mg/ml	*Echerichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogenes*
128	10.0+-0.5	9.0+-1.0	10.3+-0.5	9.0+-0.5
64	9.6+-1.0	8.6+-1.0	10.0+-0.5	8.6+-0.5
32	9.0+-1.0	8.3+-1.1	9.3+-0.5	8.3+-1.0
16	8.6+-0.5	8.0+-0.5	9.0+-1.1	8.0+-1.0
8	8.3+-0.5	7.6+-0.5	8.6+-1.1	7.6+-0.5
4	8.0+-1.1	7.3+-0.5	8.3+-1.0	7.0+-1.1
2	7.6+-1.0	7.0+-1.0	8.0+-0.5	6.6+-1.1
1	7.3+-0.5	6.6+-0.5	7.3+-0.5	6.3+-0.5
0.5	6.6+-0.5	6.3+-1.1	7.0+-1.0	6.0+-0.5
0.25	6.3+-1.0	6.0+-0.5	6.6+-1.0	5.6+-1.1
Genfamyein 10 mg/disc	8.0+-0.5	8.3+-1.0	9.0+-0.5	7.0+-0.5
Negative Control DMSO 100%	0.0+-0.0	0.0+-0.0	0.0+-0.0	0.0+-0.0

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