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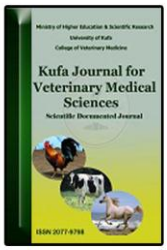

In vitro antifungal activity of extracts of Anastatica Hierochuntica

Assistant lecturer: Wedad Salih Daoowd

Department of Biology, College of Vet. Medicine, University of Diyala
E-Mail: Biology-microbiology@ymall.com

Abstract:

The antifungal activity of aqueous and organic(acetone and methanol) leaf extracts of Anastatica hierochuntica was evaluated a gainst some common pathogenic fungi using the paper disc diffusion method .ALL the extracts were active against the test organisms with the methanol extracts showing the highest activity against candida albicans (28 mm zone of inhibition), Cryptococcus neoformans(24 mm zone of inhibition) ,fusarium oxysporum and penicillium digitatam (18 mm zone of inhibition), followed by the acetone extracts against penicillium digitatam (16 mm zone of inhibition), at 250 mg /ml . the aqueous extracts demonstrated the lowest activity (8 mm zone of inhibition),against penicillium digitatam and (6 mm zone of in hibition) against Aspergillus niger at 250 mg /ml .preliminary phytochemical studies revealed that the leaves contained Hydrocarbonate, glocoberin ,Aminoacid ,glycoside ,Asteroids ,carbohydrate ,flavonoid (one structure: isovitexin and four structures flavonoloids, campferol ,Raminoglocozid ,Rutin ,Qurcetn) ,Alkaloids, tannins.

The activity of the extracts was stable at high temperatures and at acidic PH ,but decreased at alkaline PH .the minimum Inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the extracts ranged between 12.5- 150 mg /ml .the plant contain chemicals substances that can be used in the formulation of very potent antifungal agents that can be used for the treatment of Mycotic infections.

Key words: phytochemicals ,Antifungal activity , MIC , MFC , Mycotic infections

الفعالية المضادة للفطريات لمستخلص نبات عشبة كف مريم

وداد صالح داود

فرع الاحياء المجهرية، كلية الطب البيطري، جامعة ديالى

الخلاصة :

دراسة الفعالية المضادة للفطريات للمستخلص المائي والعضوي (الاسيتون ، الميثانول) لأوراق نبات عشبة كف مريم ضد بعض الفطريات المرضية المعروفة بأستخدام طريقة الانتشار بالأقراص . كل المستخلصات كانت فعالة ضد الفطريات، حيث اظهر مستخلص الميثانول فعالية عالية ضد candida albicans (28 mm zone of inhibition)، Fusarium oxysorum and penicillium Cryptococcus neoformans(24 mm zone of inhibition) penicillium digitatam (16 mm يتبعه مستخلص الاسيتون ضد ،digitatam (18 mm zone of inhibition) zone of inhibition، عند 250 ملغم / مل . المستخلص المائي اظهرالفعالية الاقل ضد penicillium digitatam (8 mm zone of inhibition) وAspergillus niger(6 mm zone of inhibition) عند 250 ملغم / مل .

تشير الدراسات الى احتواء اوراق النبات على المواد الفعالة :

Hydrocarbonate ,glocoberin ,Aminoacid , glycoside ,Asteroids ,carbohydrate , flavonold (one structure : isovitexin and four structure flavonoloids : campeferol .Raminoglocozide , Rutin ,qurcetn ,Alkaloids , tannins فعالية المستخلص بقيت ثابتة عند درجات الحرارة العالية والحامضية العالية لكن قلت الفعالية عند الفاعدية (انخفاض قيم PH) ، التركيز المثبط الادنى (MIC) ، التركيز الفطري القاتل (MFC) للمستخلص تراوحت بين(5 ،12-150) ملغم/ مل .

ان المحتوى الكيميائي للنبات يمكن ان يستفاد منه كمضاد فطري قوي يمكن استخدامه للاصابات الفطرية.

الكلمات المفتاحية : العدوى الفطرية ،الحدالادنى للتركيزالقاتل للفطريات ،الحدالادنى للتركيزالمثبط ، الفعالية المضادة للفطريات ،الموادالكيميائية النباتية.

Introduction:

Opportunistic fungal infections represent a significant cause of morbidity and mortality in immunocompromised paitents , including those with AIDS cancer and organ transplants (1, 2, 3)Despite the increase in fungal infections ,therapeutic options are very limited and are often unsatisfactory because of elevated toxicity and an inability to eradicate infections and in recent times ,increase in resistance to antifungal agents including Amphotericin B (AMB), Fluconazole and itraconazoleby many fungal species (4, 5) this therefore highlights the need for search of new antifungal agents with potent and broad spectrum fungicidal activities for the effective management of these infections (,6 , 7, 8) plant sources may be safer alternative sources for antifungal agents

A .hierochuntica(Family : Brassicaceae) Commonly Called Common rose of Jericho , other common names include dinosaur plant , Jericho rose , Mary,sFlowet , Mary,s hand , Palestinian tumbleweed , resurrection plant , st . Mary,s Flower , true rose of Jericho (9, 10) , The plant is asmall gray annual herb that rarely grows above 15 Centimetres (6.in high) , bears Minute White Flowers . (11,12), Anastatica is Found in arid areas in the Middle Eastsahara Desert , including Parts of North Africa regions of Iran , Egypt , Palestine , Iraq , Jordan , Pakistan (13,14) . After the rainy seaon , the Plant dries up , dropping leaves Curling branches into atight ball , hibernates" Within the ball, the fruits remain attached and closed protecting the Seeds Preventing them From being disper seed Prematurely . The seeds are Very hardy and Can remain dormant For Years . Wetted again in alatler rainy Season , the ball uncurls and the Plant Wakes up From its dormant state , which Causes the Capsular Fruits open (dehisce) to dis perse the Seeds Ifwater is sufficient the dispersd Seeds germinate within hours . (15 , 16) . This study was Carried out in order to investigate the antifungal l activity of the Plan-1 against Some Common Pathogenic Fung.

Materials and Methods:

Fresh Ieaves of A .hierochuntica were collected from the river side gardens in Iraq

Test Organisms

Candida albicans, Aspergillus niger, Penicillum digitatum , Fusarium oxysporum, Cryptococcus neoformans, Were laboratory isolates and saboraud dextrose agar (SDA) (Oxoid) and other reagents (analytical grade) and glass wares used for the purpose of this study were all obtained from the Microbiology Laboratory , Department of Microbiology , Diyala University .

Preparation of Extracts

The plant extracts Were prepared using the method earlier described by (17) with minor modification. The fresh leaves were shade dried to constant weight for 5 days , coarsely powered using mortar and pestle and further reduced to powder using electric blender and stored in closed bottles ,Twenty grames (20 g) each of the ground plant prt was soaked separately in 100 ml , water , methanol and acetone at room

temperature (30 -32 c) for 24 h with manual agitation of the flask using a sterile glass rod after every 6 h. after 24 h , each of the extracts was filtered using a clean sterile muslin cloth and then using whatman no ,1 filter paper all the extracts were concentrated using a rotatory evaporator at 40 c and then kept in the fidge prior to use .

Determination of phytochemical constituents

The freshly prepared extract was subjected to standard phytochemical analyses for different constituents such as tannins ,alkaloids ,flavonoids ,glycosides ,saponins and phenols as earlier described (18).

Assay of antifungal activity

The paper disc diffusion method as described by (5) was used with slight modification 10 ml saboraud dextrose ager (SDA) (oxoid) was dispensed into petri dishes and allowed to solidify a micropipette was used to introduce 0.1 ml of the spore or conidia suspensions adjusted to 105 – 10 7 cells/ml using haemocytometre was added on to the agar plate ,and spread with glass rod spreader under sterile conditions ,sterilized discs (6 mm , whatman no 1) were prepared by soaking in different concentrations of the extracts (50 ,100 .150 ,200and 250 mg /ml) for 6 h in bijou bottles the discs were them then removed and allowed to dry in a sterile petri dish ,then stored in screw capped bottles for further use to assay for antifungal activity ,five of the discs impregnated with different concentrations of the extract were placed on a fungal spore or conidia seeded plate with the help of sterile forceps discs soaked in sterilized distilled water only without extracts were used as control .three replicates were produced for each fungus culture plates containing c. alblcans were incubated at 37 c for 24 h while other culture plates containing the rest of the fungi were incubated at room temperature (32 _ 35 c) for 48 72 h . antifungal activity was determined by measurement of the zone of inhibition around the discs after the period of incubation

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC was determined using the agar dilution method described by(19) varying concentrations (300 .0 ,250.0.200.0150.0 100.0.50.0,25 .0 and 12 .5 mg /ml) of the extracts were prepared and incorporated into saboraud dextrose agar .the plates were incubated at 25 c for 48 h and inhibition of growth was noted the minimum inhibitory concentrations (MICs) were recorded after 48 h .

]to determine the MFC .plates which did not show any growth after 48 h from the MIC determination were further incubated for 72 h and after incubation ,the concentration at which no visible growth was seen was noted as the minimum fungicidal concentration

Effect of Temperature and PH on Antimicrobial activity of extracts

Five millilters of 250 mg/ ml of methanol extracts were constituted in test tubes and treated at 4c in the refrigerator and 60 and 100 c in a water bath for 1 h and tested for antimicrobial activity

TO determine the effect of pH, the methanol extracts (250 mg /ml) were treated at pH ranges of 2 to 10 using IN HCI and IN NaOH solutions respectively in series of test tubes for Ih. After the 1 h period of acid-base treatment, the extracts were again neutralized using 1N HCI and 1N NaOH solutions as the case may be and then tested for antifungal activity .

Results:

Table 1 .Anti fungi activity of A .hierochuntica against some Fungi :

Test organisms	Extract	Conc . (mg \ml) \ zone of inhibition (mm)				
		50	100	150	200	250
C .albicans	ME	9	18	20	26	28
	AE	6	8	10	12	14
	WE	_	_	_	6	8
Cr.neoformans	ME	8	10	18	20	24
	AE	_	8	10	12	14
	WE	_	6	8	8	12
F .oxysporum	ME	_	6	8	12	18
	AE	_	_	6	8	12
	WE	_	_	6	8	10
P .digitatum	ME	6	8	10	12	18
	AE	-	8	10	12	16
	WE	_	_	_	6	8
A .niger	ME	6	8	10	12	14
	AE	_	6	8	10	12
	WE	_	_	6	6	6

Key : WE = water extract , AE = acetone extract , ME = methanol extract

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the methanolic extracts of the plant against the test fungi

Test organism	MIC (mg/ml)	MFC (mg/ml)
A .niger	100	1 50
P .digitatum	100	150
F .oxysporum	50	50
Cr .neoformans	25	50
C .albicans	12.5	25

Discussion:

Preliminary phytochemical analysis revealed that plant possessed tannin, flavonoids and Alkaloid , Amino acid Pl ants have been know to posses bioactive constiuents as protective, substances against bacteria, fung, viruses and pests (20). Presence of tannins in the theIeaves has also been reported (21). The stem bark has been reported to contain tannins and to Posses antibacterial activity. The methanol

extracts of the plant demonstratrated the highest activity compared to the aqueouas and acetone extracts.The highest activity (28 mm zone of inhibition) was demonstrated against C. albicans followed by those aagainst Cr. neoformans (24mm zone of inhibition) at 250 mg/ml (Table 1). The demonstration of antifungal activity against C. albicans and Cr. neoformans is a further justification for the application of the leaves in the treatment of vaginal irritation discharges (22). The highest activities (14 and 16 mm zone of inhibition) was demonstrated against Cr. neofrmans and P. digitatum respectively by the acetone extracts at 250 mg/ml while the least activity (6 mm zone of inhibition) against A. niger was demonstrated by the aqueous extracts at 250 mg/ml. The activity of the extracts against these fungi also gives scientific bases on the application of the infusion of the leaves in the treatment of eczema and other skin infections (22,23). The differences observed in the activities of the various extracts exracts may by as result of their varying degrees of solubility in the different solvents. Is has been reported that different solvents have different solubility capacities for different phytoconstuents (20). The exhibition of antifungal activity against C. albicans, Cr. neoformans and the other fungi is a very significant outcome because its an indication that there is Possibility of sourcing alternative antibiotic substances in these plants for the development of newer antifungal agents that will be very effective against candidiasis (caused by C. albicans), systemic mycosis (Aspergillusspp), and crytoccous (Cr. Neoformans) that are rapidly becoming refractile to chemotheraopy with the standard antifungal agents (24,25) Table 2 showed the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts. The result showed that the MICs and MFCs values were in the range of 12.5 _150 mg/ml. The lowest MIC and MFC values of 12.5 mg/ml was demonstrated against C. albicans, while the highest MLC and MFC value of 150 mg/ml was drmonstratedA. niger Low MIC and MFC values are indication of efficacy of the plant while high MIC and MFC values are indication of inefficiency of the antifungal agents The demonstration of activity against the different category of Pathogenic fungi by leaves of A .hierechuntica an indication that the plant can be used to source newer group of antifungal substances that can be used to develop more effective antifungal agents, and also justifies its local usage for the treatment of skin infections. The plant canthere fore be used for the treatment of mycotic infections such as candidiasis, ringworm and cryptococcsis and other systemic mycoses. Further research however needs to be carriedout in order to determin the antifungal actifungal activity of the plant against a Wider group of pathogenic fungi, and to investigate its toxicological properties and also further purification of the extracts should be carried out with a view to producing safer antifungal chemotherapeutic agents for man use.

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