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Antimicrobial activity test of the crude extracts of some marine Mollusca against selected pathogenic fungi, in vitro

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Abstract

Crude extracts of marine Mollusca *Sepia* sp. ,*Tibia insulaechorab-curta* and *Thais* sp. marked as A, B and C respectively, using 50 % acetic acid were studied for their antifungal activity using agar well diffusion technique against seven fungi (*Aspergillus niger, A. flavus, Candida albicans , C. krusei, C. tropicalis , C. famata* & *C. guilliermondii*). No antifungal activity was recorded against *A. niger* &*A. flavus* in all concentrations of *Thais* sp. extract. *Sepia* sp. & *Tibia insulaechorab-curta* extracts exhibited significant differences ($P \geq 0.05$) to good antifungal activity more than *Thais* sp. extract. An extract has been showed high activity against *C. albicans* (inhibition zone=27.6 mm) diameter, and minimum zone of inhibition (16mm) was recorded against growth A. *niger* of same extract. In B extract, maximum inhibition zone (26.6mm) in growth *A. flavus* & *C. albicans* and minimum inhibition zone diameter (15mm) in growth *A. niger* .Also the minimal inhibitory concentration of all extracts were detected .The results showed the inhibition zones diameter of Fluconazole as positive control approximately with the inhibition zones of crude extracts .

1- Introduction :

Molluscs are widely distributed throughout the world and have many representatives in the marine and estuarine ecosystem, namely slugs, whelks, clams, mussels, oysters, scallops, squids and octopus (Kamboj,1999).The number of natural products isolated from marine organisms increases rapidly, and now exceeds, with hundreds of new compounds being discovered every year (Faulkner, 2002; Proksch and Muller, 2006). Large proportions of these natural compounds have been extracted from marine invertebrates, especially Molluscs, Sponges, Ascidians and Bryozoans, and some of them are used in clinical trials (Proksch, *et al*., 2002). The need for discovery of new and novel antibiotics is imperative because evidence suggests that development and spreads of resistance to any new antimicrobial agents is inevitable. From 1960, approximately 300 bioactive marine natural products were field for patent .Approximately 6,500 bioactive compounds have been isolated from the marine organisms (Kamboj,1999) .Many classes of bioactive compound exhibiting antitumour, antileukemia ,antibacterial & antiviral activities have been reported world wide (Pettit *et al*.,1991) .

The aim of the present study was, first to analyze the antifungal activity from the tissues extracts of three species of Mollusca and to determine the Minimal Inhibitory Concentration (MIC) against seven species of fungi.

Material and Methods

Collection and preparation of samples

The Molluscs *Sepia* sp., *Tibia insulaechorab-curta* and *Thias* sp. marked as A, B and C respectively were collected from Arabian Gulf water. The collected samples were extracted by 50% acetic acid (Li *et al*.,1962) , and the crude extracts (A&B) subjugated to various chemical tests(Al-hussan,2007;Degiam ,2009) , and C extract subjugated to various chemical tests too, to get acquaint chemical compound.

All fungal strains (*Aspergillus niger*, *A. flavus*, *Candida albicans* , *C. krusei* , *C. tropicalis* , *C. famata* & *C. guilliermondii*) were obtained from Microbiology laboratory of Medicine college-University of Thi-Qar that were diagnosed by Traditional Laboratory methods (Morphology and Biochemical tests)(McGinnis,1980;Finegold & Baron, 1990).

Antimicrobial activity assay:

Mollusca crude extracts were tested, in vitro against fungal growth. The antifungal activity of the sample was assayed by the standard agar well diffusion technique (Nathan *et al* .,1978 ;NCCLS,1998). All the fungal strains were inoculated in SDA at 28°C for (1-3 days) . 300 mg of lyophilized powder was dissolved in 1ml of water ,then 100 µL of the extract was pipetted on a 6mm well . The plates were incubated at 28°C. Zones of inhibited fungal growth were observed as clear halos (zones) around the well . Antifungal activity was measured as diameter of zone of inhibition, excluding the well diameter. Water was used as negative control (-), and Fluconazole was used as positive control (+) with concentration 50 mg/ml .

Determination of Minimal Inhibitory Concentration (MIC)

To determine minimal inhibitory concentrations of all extracts, using 5 concentrations: 200, 100, 50, 25 and 12.5 mg/ml from stock solution (300mg/ml) by using agar well diffusion technique (Nathan *et al*.,1978;NCCLS,1998). Preparing fungal dilutions through comparison with

McFarland tube 1 (3×10^6) (Hammer *et al*., 2002).

The data were analyzed using X^2 and P-value to show significant differences at ($p \geq 0.05$) in comparison with positive control.

Results:

Sepia sp. , *Tibia insulaechorab-curta* & *Thais* sp. extraction showed that the *Thais* sp. extract contains Amino acids, Proteins , Saponins &Carbohydrates, but the extract had no Aldehyde ,Ketons ,Flavones Alkaloids and Glycosides while the *Tibia insulaechorab-curta* extract contained Proteins , Amino acids and Carbohydrates only ,whereas *Sepia* sp. extract contained Amino acid, Proteins, Saponins ,Carbohydrates ,Aldehydes,Ketons,Flavones and Alkaloids .

Antimicrobial assay:

All the results of antifungal activity are shown in Table (1). *Tibia insulaechorab-curta* extract, the activity were 26.6mm diameter of zone of inhibition growth against *A. flavus* and *C. albicans* ,and minimum inhibition zone (15mm) in growth *A. niger* . *Sepia* sp. extract, the maximum inhibition zone 27.6mm in *C. albicans* and (16mm) in *A. niger* growth, while *Thais* sp. extract, there was antifungal activity observed in *A. niger* and *A. flavus* growth, whereas showed antifungal activity on all testes yeasts growth and inhibition zones ranging from (8 -15.3mm) . From the X^2 and P-value showed significant differences on fungi *A. Niger, A. flavus* and *C. guilliermondi* at ($p \geq 0.05$) in comparison with positive control .

Table (1) Diameters of inhibition zones (mm) of the antifungal activity of *Sepia* sp. , *Thais* sp. and *Tibia insulaechorab-curta* extracts

Fungi	B	C	A	Control (-)	Control (+)	X^2	P-value
A. niger	15	-*	16	-	25	20.68	≥ 0.001
A. flavus	26.6	-	20	-	27	24.14	≥0.001
Candida albicans	26.6	13.3	27.6	-	27	6.516	=0.08
C. tropicalis	26	14.6	22.6	-	29	4.67	=0.197
C . famata	23	15.3	26.3	-	29	4.66	=0.19
C. krusei	23.3	15.3	20	-	29	4.72	=0.0193
C. guilliermondii	22	8	23.3	-	30	12.27	>0.01

*Absence of the antifungal activity

The minimal inhibitory concentration of crude extracts:

The results of minimal inhibitory concentration (Table 2) showed that the MIC of C extract was 200 mg/ml on all yeasts growth and 300 mg/ml on moulds growth. The MIC of B extract was 100 mg/ml on *A. niger* , *A. flavus* ,*C. tropicalis* , *C. famata* &*C. krusei* , and was 50mg/ml on *C. albicans* & *C. guilliermondii*. But the MIC of A extract ranged from 100 mg/ml on *A. niger* & *A. flavus* to 50 mg/ml on *C. albicans*, *C. tropicalis*, *C. krusei* & *C. guilliermondii* to 25 mg /ml on *C. famata* .

Table (2) The minimal inhibitory concentration of A, B &C extracts

Fungi species	MIC (mg/ml)		
	A	C	B
Aspergillus niger	100	300	100
A. flavus	100	300	100
Candida albicans	50	200	50
C. tropicalis	50	200	100
C .famata	25	200	100
C . krusei	50	200	100
C. guilliermondii	50	200	50
L.S.D	0.049	1.85	0.0341

The shadow fields refer to significant differences at ($p \geq 0.05$)

Discussion:

Mollusca tissues were extracted by 50 % acetic acid, because this acid has the ability to lyses animal tissues (Al-hussan, 2007). *Thais* sp. extract contains Amino acids, Proteins, The marine animals contains Proteins, Peptides (Baslow, 1977), Dolastatin, a cytotoxic peptide from *Dolabella auricularia* is an antineoplastic substance (Pettit *et al*., 1989). The extract contains Saponin & Carbohydrate was isolated from *Lobophytum* spp. collected from Hainan Island (He *et al*., 2002), the tissues of *Crassostera virginica* & *Mercenaria mercenaria* contain Carbohydrates substance (Baslow, 1977).

In the present investigation a pronounced antimicrobial activity had been observed against some fungal strains, Similar result was reported in four bivalves against few pathogens and found that extracts showed significant activity against *Bacillus subtillus* (Jaysali *et al*.,2001) . The presence of antimicrobial activity in Mollusca had been reported from the whole body meats of Mollusk, Gastropoda *Trochus tentorium* was extracted with various solvents (Anbuselvi *et al*., 2009). Prem Anand and Patterson Edward (2002) reported moderate antibacterial and antifungal activity from extracts of various bivalve Mollusks.

In this study, wide antifungal activity has been recorded in all extracts which is the significant finding of the study ,and showed different antifungal activity, possibly due to presence of different chemical substance in extracts , the biological activity of crude extracts, possibly due to the *Sepia* sp. extract is proline rich extract (Degiam,2009). Cyclic heptapeptide, stylisin 1 proline-rich cyclopolypeptide exhibited moderate to good antimicrobial activity against gram negative bacteria *Klebsiella pneumoniae* , *Pseudomonas aeruginosa*, dermatophytes

and *Candida albicans* with minimum inhibitory concentration (MIC) of 6µg/ml (Dahiya *et al*., 2009). But the *Tibia insulaechorab-curta* extract contain cystein which have antimicrobial activity (Al-hussan, 2007) , Two antimicrobial peptide defensins A & B isolated from the blood of immune challenged *Mytilus edulis* , a family of cysteine-rich cationic peptides (Charlet *et al*., 1996) .Whereas *Thais* sp. extract was contained protein and amino acid ,to get its biological activity against microorganisms .

Extract of Mollusk, *Drupa margariticola,* exhibited higher antibacterial activity against human pathogens (Chellaram and Edward, 2009).Which is in agreement with the present work. Polysaccharide isolated from Cuttlebone *Sepia aculeate* and *Sepia brevimana* have broad spectrum against 9 bacterial species and 4 fungi (Shanmugam *et al*.,2008) . Latrunculin-A from *Chromodoris elisbenthina* that showed inhibition activity against *C. albicans* (Okuda & Sheuer, 1985). But there are some reports on the isolation of the antifungal compound from marine animals such as general: puphenone from marine sponges (Amede & Chevolot, 1982) and Dolastain-10 from tunicate and spongistatin from sponges (Pettit *et al*., 1998 a,b) .

Thais sp. extract showed no effect on molds *A. niger* & *A. flavus* and showed the MIC of same extract was high, Ronald (1997) has reported that the fungi are more resistant than the bacterial strains to the tested compound, this could be lead to the nature of fungal cell wall made up of chitin. The hard cover of the exoskeletons of the arthropods are also made up of chitin, which is relatively resistant, including microbial decomposition.

In conclusion in the present study, the crude extracts have been extracted from Mollusca showed promising antifungal activity against human pathogenic fungi. This finding is very significant for the discovery of new potent drugs against these pathogenic fungi .

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اختبار الفعالية الضد مايكروبية للمستخلصات الخام لبعض النواعم البحرية تجاه بعض الفطريات الممرضة ،مختبريا

زينب داخل دغيم

قسم الأحياء المجهرية- كلية الطب- جامعة ذي قار

المستخلص

تم دراسة الفعالية المضادة للفطريات لمستخلصات النواعم البحرية الخام .*Sepia* sp و-*Tibia insulaechorab curta* و.*Thais* sp رمز لها A و B و C على التوالي باستخدام ٥٠% حامض الخليك وباستخدام تقنية الحفر تجاه سبعة أنواع فطرية : *Aspergillus niger* و *A. flavus* و *Candida albicans* و *C. krusei* و *C. tropicalis* و *C. famata* و *C. guilliermondii*. لم يسجل مستخلص *Thais* sp. أي فعالية مضادة للفطريين *A. niger* و *A. flavus* في جميع التراكيز المستخدمة من المستخلص . أظهر المستخلصان -*Sepia* sp. & *Tibia insulaechorab curta* تقاربا في فعاليتهما المضادة للفطريات عند مستوى احتمالية ($P \geq 0.05$) أكثر من المستخلص .*Thais* sp. أظهر المستخلص A أعلى فعالية حيوية تجاه *C. albicans* بقطر تثبيط (٢٧,٦ ملم) وأقل منطقة تثبيط (١٦ ملم) على نمو *A. niger* . أظهر المستخلص B أعلى منطقة تثبيط (٢٦,٦ ملم) على الفطر *C. albicans* و *A. flavus* واقل منطقة تثبيط (١٥ ملم) على نمو الفطر *A. niger* . كما تم تحديد التركيز المثبط الأدنى لجميع المستخلصات . أوضحت النتائج أن أقطار تثبيط Fluconazole كسيطرة موجبة تتقارب من أقطار تثبيط المستخلصات الخام .