

Antimicrobial activity of some crude marine Mollusca extracts against some human pathogenic bacteria

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ABSTRACT :

The antimicrobial activity of three species of marine Mollusca extracts, Sepia sp. ,Loligo sp. and marine snail Tibia insulaechorab-curta marked as S, L and T respectively were studied against five bacterial species of family Enterobacteriaceae (Escherichia coli, Pseudomonas aeruginosa , Klebsiella oxytoca , Proteus mirabilis and Serratia liquefaciens) that diagnosed by API 20 E technique . S & T extracts exhibited significant differences to good antimicrobial activity more than L extract . All bacteria were tested have shown susceptible to S and T extracts, Antibacterial sensitivity test of 6 antibacterial antibiotic showed the crude extracts clear effect more than the antibacterial antibiotic. The minimal inhibitory concentration of all extracts were detection .

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INTRODUCTION:

The Mollusca are animals belong to the phylum Molluscs ,there are around 93,000 recognized species extant, making it the largest marine phylum with about 23 % of all named marine organisms. Representatives of the phylum live in a huge range of habitats including marine, freshwater and terrestrial environments. Molluscs are a highly diverse group, in size, in anatomical structure, in behaviour and in habitat (Haszprunar,2001).The marine environment comprises of complex ecosystem with a plethora of organisms and many of these organisms are known to possess compounds as a common means of defense (Indap & Pathare,1998) .The organisms from marine environment have been found possessing a vast array of new pharmaceutical compound with novel activities that

will provide new drug leads to compact microbial pathogens currently developing resistance to conventional antibiotic therapies . The study of natural products that exhibit biological activity, derived from plants and animals has long been showing significant biomedical value and crude products isolated from marine organisms have served as source of many drugs (Kamboj,1999).In the most of the publications concerning antimicrobial activity in Mollusca, either single body compartment alone, like haemolymph and egg masses, or extracts of whole bodies have been tested for activity (Haug et al .,2003). Further the cuttlefish also reported antibacterial and antifungal activities against some of the human pathogenic microorganisms (Rajaganapathi, 2001).The ocean served just not only as the source of antibiotic but it is indeed reservoir of other bioactive

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compounds too (Yamazaki,1993). The aim of this study was the investigating the antibacterial activity of *Sepia* sp., *Loligo* sp. and *Tibia insulaechorab-curta* extracts against five species of bacteria .

MATERIAL AND METHOD:

Extraction of *Sepia* sp. , *Loligo* sp. and *Tibia insulaechorab-curta*

Homogenize *Sepia* sp. , *Loligo* sp. and *Tibia insulaechorab-curta* tissues (whole body) in electrical blender with an equal volume of 50% acetic acid , and the homogenate was put on Magnetic stirring for 24 hour , The pH was adjusted to 5.0 using pH meter, Centrifuge at 2000 g/h and remove sediment .Dialyze against distilled water for 48 hour was carried out (in refrigerator) and the water was replaced each that 6 hour and lyophilize by Freeze drier (Li et al .,1962) .

Qualitative chemical test

Crud extracts subjugate to varies chemical tests to get acquaint chemical compound for S, L (Degiam ,2009) and T (Al-hussan ,2007) .

Bacterial strains

Five bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa* , *Klebsiella oxytoca* , *Proteus mirabilis* and *Serratia liquefaciens*) were used for studying the antibacterial activities of cuttlefishes and marine snail extracts . Strains were obtained from Al-Hussain Teaching Hospital , and diagnosis by API 20E (Analytical profile index 20 Enterobacteriaceae) .

Antibacterial assay

In vitro antibacterial activity was determined by agar well diffusion technique (Perez et al.,1990) , 100 mg of lyophilized powder was dissolved in 1ml of water . This agar plates were

incubated at 37 °C for 24 hours . Three replicates were formed for every bacterium (Cruickshank et al .,1975) ,inhibition zone was measured (mm) . Antimicrobial antibiotics disk : ,Amikacin (AM), Carbenicillin (Py) ,Tapromycin and Ciprofloxacin(Cip), Erythromycin (E) and Gentamicin (CN) were used as antibacterial standards .

Determination of the minimal inhibitory concentration

To determine the MIC of all extracts ,using 3 concentration 50,30 and 10 mg/ml from stock solution (100mg/ml) by using agar well diffusing technique

RESULTS:

Extraction and Qualitative chemical test :

Sepia sp. , *Loligo* sp. and *Tibia insulaechorab-curta* were extraction using 50 % acetic acid, because the later has ability to lyses animals tissues and determined the chemical compounds The S and L extracts contains Proteins ,amino acid, Saponins, carbohydrate, Aldehyde & Keton , Flavones and alkaloids , but The T extract contain protein , amino acid and carbohydrate only .

Antibacterial activity :

The results of antimicrobial activity (Table 1) . In the present study ,high antibacterial activity was found on all bacterial strains of S,T and L extracts ,also showed signification variation in the inhibition zone .At S extracts ,the highest activity was recorded through 23.6 mm inhibition zone against *Pseudomonas aeruginosa* and the lowest activity was recorded against *Proteus mirabilis* with inhibition zone 17.5mm , and at T extract showed approach activity of S extract , but L extract showed different inhibition zone , L extract was ranging from 11 to 18 mg/ml on all bacterial strains . In this study showed the L extract has

antibiotic activity less than S and T extracts .

In this case of control (E, CN& Tab) antibacterial antibiotic no activity record against any bacterial strains ,while Cip, Am & Py have activity in Escherichia coli , Klebsiella oxytoca, Serratia liquefaciens, but all antibacterial antibiotic do not effect on Pseudomonas aeruginosa, whereas showed the S & T extracts have large effected on same bacteria and other bacterial strains . Antimicrobial activity of Cephalopoda extracts were compare with antibacterial antibiotics ,showed the extracts have significant activity more than antibacterial antibiotics

The minimal inhibitory concentration of crude extracts S,L& T

The result of minimal inhibitory concentration (Table 3) showed , The MIC of S extract was 10 mg /ml on Pseudomonas aeruginosa, Escherichia coli and Serratia liquefaciens whereas equal 30 mg/ml on Proteus mirabilis and Klebsiella oxytoca . MIC of L extract was ranging from 30 mg/ml on Pseudomonas aeruginosa to 50 mg/ml on other bacterial types ,while the MIC of T extract was 10 mg /ml on all bacterial exception MIC on Proteus mirabilis that has equal 30 mg/ml .

DISCUSSION :

In recent years, great attention has been paid to a study the bioactivity of natural products of their potential pharmacological utilization. Among these 50 have found widespread use in the prevention and treatment of bacterial disease in animal and man (Gale, & Kiser, 1967) . The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950s (Nigrelli et al ., 1959) Since this time, a large number of marine organisms from a wide range of phyla have been screened for anti

microbial activity (Shaw et al ., 1976) . Many of these organisms have been antimicrobial properties, although most of the antibacterial agents that have been isolated from marine sources have not been active enough to complete with classical anti microbial obtained from microorganisms (Rinehart et al .,1981) . A screening of antibacterial activity in cuttlefishes extracts of Sepia sp. and Loligo sp. and marine snail of Tibia insulaechorab-curta were conducted . The results show that in vitro antibacterial in cuttlefishes and marine snail extracts .Antibacterial activity has previously been described in wide range of mollusk species (Shanmugam,2008 ;Benkendorff et al ,2001; Gunthorpe & Cameron,1987; Constantine et al,1975) .

In this study, wide spectral antibacterial activity has been recorded in almost all the extracts which is the significant finding of the study .(Kagoo & Ayyakkannu, 1992) reported that hypobranchial gland of Chicoreus ramosus exhibited a broad spectral activity against 10 bacterial strains . The Broad spectrum of biological activity of crude extracts, Because of the S & L extracts are proline rich extracts (Degiam, 2009), But the T extract contain amino acid cystein which have antimicrobial activity (Al-hussan, 2007) .To compare the biological activity of crude marine extracts and antibacterial antibiotic showed the extracts were clear effect more than the antibacterial antibiotic . Only very few studies have been carried out on the antimicrobial activity of the internal shell of cuttlefishes, but many such studies are available for the extracts from the whole body tissue whose results could be compared with that of the present study (Jayaseeli et al .,2001) when study the antibacterial activity of Donax faba, D.modesta & Circe scripta agaist nine pathogenic bacteria such as

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S. aureus, *K. pneumoniae*, *E. coli*, *P. mirabilis*, *P. vulgaris*, *S. flexneri*, *S. typhi*, *B. subtilis* & *V. cholerae* reported broad spectrum antibacterial activity for the water and heptane extracts. In conclusion in the present

study indicates that the good source of antibacterial activity agents and would replace the existing inadequate and cost effective antibiotics.

Table (1) Diameters of inhibitions zone (mm) of the antibacterial activity of *Sepia* sp., *Loligo* sp. and *Tibia insulaechorab-curta* extracts

	S	L	T
<i>Escherichia coli</i>	19.3	17	18.3
<i>Pseudomonas aeruginosa</i>	23.6	18	22.3
<i>Proteus mirabilis</i>	17.5	11	14.6
<i>Klebsiella oxytoca</i>	18.3	11.3	22.6
<i>Serratia liquefaciens</i>	21	12.6	22.3
L.S.D	2.045	1.504	2.467

Table (2) Diameters of inhibitions zone (mm) of the antibacterial activity of 6 antibacterial antibiotics

	Cip	E	Py	AM	Tab	CN
<i>Escherichia coli</i>	25	-	-	15	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-
<i>Proteus mirabilis</i>	-	-	-	15	-	-
<i>Klebsiella oxytoca</i>	30	-	-	22	-	-
<i>Serratia liquefaciens</i>	30	-	23	-	-	-

Absence of the antimicrobial activity *

Table (3) The minimal inhibitory concentration of S,L & T extracts

	MIC (mg/ml)			Inhibition zone (mm)		
	S	L	T	T	L	S
<i>Escherichia coli</i>	10	50	10	7.6	8.3	8.3
<i>Pseudomonas aeruginosa</i>	10	30	10	8.6	8	11.3
<i>Proteus mirabilis</i>	30	50	30	10	8	10.3
<i>Klebsiella oxytoca</i>	30	50	10	10.6	11	9.3
<i>Serratia liquefaciens</i>	10	50	10	7.3	8.3	8
L.S.D	0.041	2.007	0.039	0.046	1.988	0.0374

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الفعالية الحيوية لبعض مستخلصات النواعم البحرية الخام تجاه بعض البكتريا الممرضة للإنسان

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الخلاصة

تم دراسة الفعالية المضادة للجراثيم لثلاث أنواع من مستخلصات النواعم البحرية الخام *Sepia* sp. و *Loligo* sp. و *Tibia insulaechorab-curta* و رمز لها S و L و T على التوالي تجاه ٥ أنواع بكتيرية من العائلة المعوية (*Escherichia coli* و *Pseudomonas aeruginosa* و *Serratia liquefaciens* و *Proteus mirabilis* و *Klebsiella Oxytoca* المشخصة بتقنية API 20 E . أظهر المستخلصان S و T تقارباً في فعاليتهما المضادة للجراثيم أكثر من المستخلص L ، كما أظهرت كل الأنواع البكتيرية حساسية تجاه المستخلصان S و T ، ولوحظ في اختبار الحساسية الدوائية لست مضادات بكتيرية أن المستخلصات الخام ذات تأثير أكبر من المضادات البكتيرية . كما تم تحديد التركيز المثبط الأدنى لجميع المستخلصات .

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