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## Antibacterial activity of the whole body extract of marine mollusca (*Cypraea* sp.) and edible oyster (*Saccostrea cucullata*)

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### Abstract

The present study was carried out to the antibacterial properties of the whole body extract of two species *Cypraea* sp. And *Saccostrea cucullata* against five enteric human pathogen collected from Frazergunj, Sundarban, Fish herber area of West Bengal and Digha. The marine environment is an exceptional reservoir for bioactive natural products. The marine environment comprise of complex ecosystem with a plethora of organisms and many of these organism are known to possess bioactive compounds as a common means of defense. The marine natural products have been investigated predominantly for their antimicrobial, cytotoxic antitumor and anti-inflammatory properties. The present study based on the following – 1. To ascertain the antibacterial activity of extract from locally available edible oyster (*Saccostrea cucullata* from Frazergunj, Sundarban, Fish herber) against five pathogen. 2. To ascertain the antibacterial activity of extract from marine mollusca (*Cypraea* sp from Digha) against five human pathogen. 3. To determine the nature of bioactive compounds of the whole body extract from marine mollusca and edible oyster.

**Keywords:** Antibacterial activity, *Cypraea* sp. *Saccostrea cucullata*

### Introduction

Ocean offers a large biodiversity of fauna & flora which is estimated to be over 50,000 species more than double of the land species. There are approximately 5,000 species of sponges, 11000 species of cnidarians, 9,000 species of annelids, 66,535 species of mollusks, 50,000 species of gastropods, 15,000 species of bivalves and 600 species of cephalopods have been reported to occur. Oyster are bivalve (two shelled) soft bodies mollusks. The Indian Sundarban is broadly divided into three salinity regions, out of which the high saline zone supports the oyster bed in the intertidal regions. In India, common oyster species are *Saccostrea cucullata*, *Crassostrea madrasensis*, *Crassostrea gryphoides*, *Crassostrea rivulasis* and *Crassostrea discoidea*. Out of these five dominant species the first three species are very common in the Indian Sundarban.

### Materials and methods

1. Sample collection – Marine mollusca (*Cypraea* sp.) and Edible Oyster (*Saccostrea cucullata*) samples were collected from Digha and Frazergunj, Sundarban, Fish herber
2. Extraction - *Saccostrea cucullata* was collected from Frazergaunge & bought to the laboratory. The shells were broken and tissue sample were washed with distilled water. Extraction of bioactive compounds from the tissue sample was done with water, ethanol, methanol, acetone, hexane, butanol, ethyl acetate and dichloro methane. To 5g of tissue sample, 5ml of water and solvent extract were centrifuged well with mortar and pestle. Water and solvent extract were centrifuged at 15,000 rpm for 30 min and supernatant were stored at – 20° C.
3. Antibacterial activity of Edible oyster extract (*Saccostrea cucullata*) – Five species of pathogenic bacteria namely *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Streptococcus faecalis* were used to screen the antibacterial activity of the edible oyster extracts. Pathogenic bacterial stains were inoculated in

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sterile nutrient broth and incubated at 37° C for 24 hours. Culture of the strains was added to the mixtures in such way the total inoculum load was ca. cells / ml. The pathogens were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No. 1 filter paper with 6mm diameter). The plate were 37° C for 24 h and antibacterial activity was measured accordingly best on the inhibition zone around discs impregnated with oyster extract (*Saccostrea cucullata*).

4. Extraction of antibacterial compounds from marine mollusca (*Cypraea* spp.) *Cypraea* spp collected from digha brought to the laboratory. The shells were broken and the tissue samples were washed with distilled water. Extraction of bioactive compounds from the tissue sample was done with water, ethanol, methanol, acetone, hexane, butanol, ethyl acetate and dichloro methane. To 5g of tissue sample, 5ml of water and solvents were added and ground well with mortar and pestle. Water and solvent extracts were centrifuged at 15,000 rpm for 30min and the supernatants were stored at -20°c until use.
5. Antibacterial activity of Marine mollusca extract (*cypraea* sp.) - Five species of pathogenic bacteria namely *Escherichia coli*, *klebsiella pneumonia*, *Staphylococcus aureus*, *Shigella dysentery*, *Streptococcus faecalis* were used to screen the antibacterial activity of the marine mollusca extracts. Pathogenic bacterial strains were inoculated in sterile nutrients broth and incubated at 37° C for 24h. Culture of the strain was added to the mixtures in such way the total inoculum load was ca.10 cells/ml. The pathogens were swabbed on the surface of the Muller Hinton agar plates and discs impregnated with the 20µl of marine mollusca extracts were placed on the surface. The plates were incubated at 37°C for 24h and the antibacterial activity was measured accordingly best on the inhibition zone around disc impregnated with oyster extract (*Cypraea* sp.)

**Results & Discussion**

Antibacterial activity of extracts from *Cypraea* sp. And *Saccostrea cucullata* are present in table 2 and 3 respectively. Antibacterial activity of different solvent (control) are presented in table 1. A comparatively account (%) of elevation of antibacterial activity of extract from *Cypraea* sp. And *Saccostrea cucullata* against control are presented in table 4&5 respectively.

**Table 1:** Antibacterial activity (mm) of different solvent (control)

Name of pathogen	Zone of inhibition (mm)							
	W	E	M	A	H	B	EA	DM
<i>E.coli</i>	6	7.3	6.3	7.3	6	9.3	8.3	6
<i>S.aureus</i>	6	8	10	6.3	6.3	8.3	7	6
<i>S. dysentery</i>	6	7.7	7	7.9	6.3	8.4	8	6
<i>k.pneumoniae</i>	6	8.6	8.5	6.3	6	9	6.3	6
<i>S.faecalis</i>	6	8	6.3	8.5	6	8.5	7.2	6

[w –Water, E – Ethanol, M – Methanol, A-Acetone, H-Hexane, B- Butanol, EA- Ethyl acetate, DM- Dichloro methane]

**Antibacterial activity of extracts from marine mollusca (*Cypraea* sp.)**

Effect of extract from *Cypraea* sp. On tested pathogenic bacteria revealed that the highest activity was noticed against *K.pneumoniae* (12.3mm) with acetone extract. Only

butanol extract showed antibacterial activity against five pathogen but other solvent have no similar type of activity. As noticed in *Cypraea* sp. Both water and ethanol extract showed no activity against five pathogen expect *E.coli*. The lowest activity (Trace) was found with dichloromethane, hexane extract against *S.aureus* and *E.coli*. Among the bacteria tested *S.dysentery* was highly resistant to most of the solvent extract expect butanol

**Table 2:** Antibacterial activity of marine mollusca (*Cypraea* sp.)

Name of pathogen	Zone of inhibition (mm)							
	W	E	M	A	H	B	EA	DM
<i>E.coli</i>	-	10.6	11	10	7	12	11	10.6
<i>S.aureus</i>	-	-	10	12	9	11	11	8
<i>S. dysentery</i>	-	-	7	-	-	10	-	-
<i>k.pneumoniae</i>	-	-	10	12.3	10.3	10	11.3	-
<i>S.faecalis</i>	-	-	10	10	-	10	-	-

[w –Water, E – Ethanol, M – Methanol, A-Acetone, H-Hexane, B- Butanol, EA- Ethyl acetate, DM- Dichloro methane]

**Antibacterial activity of extracts from edible oyster (*Saccostrea cucullata*)**

Antibacterial activity of *Saccostrea cucullata* (edible oyster) revealed that butanol and acetone extract showed highest activity against *Escherichia coli* (12mm) respectively. Only butanol extract showed antibacterial activity against maximum pathogen expect *S.dysentery*, compare to other solvent extract showed no activity against five pathogen. The lowest activity (Trace) was found with ethanol, methanol and hexane against *E.coli* (8mm), *Shigella dysentery* (8mm) and *Streptococcus faecalis* (9mm) respectively. Among the bacterial tested *Klebsiella pneumonia* was highly resistant to most of the extract expect butanol (table3).

**Table 3:** Antibacterial activity Edible oyster (*Saccostrea cucullata*)

Name of pathogen	Zone of inhibition (mm)							
	W	E	M	A	H	B	EA	DM
<i>E.coli</i>	-	8	11	-	-	12	-	-
<i>S.aureus</i>	-	-	-	12	-	10	-	-
<i>S. dysentery</i>	-	10	-	-	-	-	-	-
<i>k.pneumoniae</i>	-	-	-	-	-	10	-	-
<i>S.faecalis</i>	-	-	-	-	7	10	9	-

[w –Water, E – Ethanol, M – Methanol, A-Acetone, H-Hexane, B- Butanol, EA- Ethyl acetate, DM- Dichloro methane]

**Comparative account (%) of elevation of antibacterial activity of *Cypraea* sp. against control**

It was showed that antibacterial activity acetone extract elevated by 37%, 90%, 95%, 18% against *E.coli*. *S.aureus*, *k. pneumonia*, *S.faecalis* respectively compared with control (table4). On the other hand antibacterial activity of dichloromethane, acetone, butanol, ethyl acetate and methanol Extract were elevated as 76%, 90%, 79% and 58% against *E.coli*, *S.aureus*, *S.dysentery*, *K.pneumoniae*, *S.faecalis* respectively compared with control (table4).

Table 4

Name of pathogen	Solvent (%)							
	W	E	M	A	H	B	EA	DM
<i>E.coli</i>	-	42.20	74.60	36.98	16.66	29.03	32.53	76.66
<i>S.aureus</i>	-	-	-	90.47	42.85	32.53	57.14	33.33
<i>S. dysentery</i>	-	-	-	-	-	19.04	-	-
<i>k.pneumoniae</i>	-	-	17.64	95.23	71.66	11.11	79.36	-
<i>S.faecalis</i>	-	-	58.73	17.64	-	13.63	-	-

[w –Water,E – Ethanol,M – Methanol,A-Acetone,H-Hexane,B- Butanol,EA- Ethyl acetate,DM- Dichloro methane]

#### Comparative account (%) of elevation of antibacterial activity of *Saccostrea cucullata* against control

After solvent extract treatment it was showed that maximum antibacterial activity of (butanol,ethanol,acetone,ethyl acetate)extract were elevated

by 29%,25%, 96%,25%, against *E.coli*, *S.faecalis*, is respectively compared with control (table5). On the other hand antibacterial activity of ethanol,hexane,butanol,were elevated significantly as 9.5%,5%,16.5%,11%,20% and 13% against *E.coli*, *S.faecalis*,*K.pneumoniae*,*S.aureus*.

Table 5

Name of pathogen	Solvent (%)							
	W	E	M	A	H	B	EA	DM
<i>E.coli</i>	-	9.58	-	-	-	29.032	-	-
<i>S.aureus</i>	-	-	-	96.47	-	20.48	-	-
<i>S. dysentery</i>	-	25	-	-	-	-	-	-
<i>k.pneumoniae</i>	-	-	-	-	-	11.11	79.36	-
<i>S.faecalis</i>	-	-	-	-	16.66	13.63	25	-

[w –Water,E – Ethanol,M – Methanol,A-Acetone,H-Hexane,B- Butanol,EA- Ethyl acetate,DM- Dichloro methane]

#### Discussion

In the present investigation,distinct antibacterial activity was observed against almost all the pathogenic bacteria. Butanol extract of *Cypraea* sp. Showed highest activities against five pathogens.Acetone and Butanol extract of *Saccostrea cucullata* showed highest activity against five pathogens.It was showed that maximum antibacterial activity of Butanol extract of *Cypraea* sp were elevated by against *E.coli*, *S.aureus*,*K.pneumoniae*,*S faecalis* respectively(table4).From the discussion, we conclude that the solvent extract of marine mollusca(*Cypraea* sp.) have much more antibacterial activity than edible oyster (*Saccostrea cucullata*).Through commercial production antibiotics are highly effective to kill the bacteria used in the present study showed significant antibacterial activity compare with other solvent extraction.

#### Conclusions

Commercial antibiotic are highly effective to kill bacteria involved common infection.Acetone,Butanol,extract of marine mollusca and edible oyster used in the present study showed significant antibacterial activity compare with other solvent extraction. Butanol extract of *Cypraea* sp. Showed highest activity against five enteric human pathogen.Therefore Butanol is an ideal solvent to purify the antibacterial compound.Acetone extract and Butanol extract of *Saccostrea cucullata* showed highest activity against five enteric human pathogen.Therefore Butanol and Acetone is an ideal solvent to purify the antibacterial compound from *Saccostrea cucullata*.Antibacterial activity of marine mollusks (*Cypraea* sp.) is much more than that of edible oyster(*Saccostrea cucullata*).It is worthy to use the natural products by the coastal people because it has antibacterial activity against enteric human pathogen.

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