Screening on Antibacterial activity of mucus from freshwater snail, *Bellamya bengalensis*

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Abstract

Among the freshwater edible snail *Bellamya bengalensis* has potential nutritional and medicinal importance. Especially people suffering with anaemia, malformation of bone structure, treatment of eye problems, consume its meat and soup with belief to restore their health. Standard disc diffusion method was carried out for screening of antibacterial activities. The *Escherichia coli* showed maximum susceptibility of (14.66±57mm) inhibition zone and ciprofloxacin drug used as + control and it showed (16.66±0.58mm) inhibition zone. *Staphylococcus. aureus* showed (12.66±57) and *Proteus vulgaris* showed(12.33±58) inhibition zone respectively . Statistical analysis showed that *E.coli*, *S. aureus* and *P.vulgaris* had lesser antibacterial efficacy than +ve control ($P<0.05$, $P<0.001$ and $P<0.01$) at 5%, 0.1% and 1% respectively. Statistical analysis also showed that mucus from *B.bengalensis* had more antibacterial efficacy in *E.coli* than *S.aureus* and *P.vulgaris* ($P<0.05$). In both bacteria, *S. aureus* and *P.vulgaris* showed no significant difference, both showed same efficacy, mucus also showed an increase in antibacterial activity with increase in concentration. Therefore, they can be use to treat *E.coli*, *S.aureus* and *P.vulgaris* pathogenic infections and it will be boon for our society.

Keywords: *Bellamya bengalensis*, antibacterial activity, mucus of snail, ciprofloxacin drug.

Introduction

The freshwater edible snail *B. bengalensis is* abundantly found in all types of temporary and permanent water bodies of Jharkhand.

Ethno medicinal survey in these areas were done and was reported that since immemorial time , people are very conscious about the ethno-medicinal value of *B. bengalensis*. They strongly believe about the function of *B. bengalensis* which can cure several diseases such as controlling conjunctivitis , night blindness , diarrhoea , stomach disorder , arthritis , joint pain, rheumatism cardiac diseases controlling blood pressure , asthma , rickets (calcium metabolism) nervousness and giddiness etc. *B. bengalensis*, the common banded pond snail of India was described by Annandale *et al.*(1921)[1] and also by Sewell (1921)[2] who dealt with the ecology and growth rate. According to Srivastava, P.*et al.(*2009)[3] it breeds throughout year reaching its peak during April to July. These snails breed all the year round and lay the eggs on the lower surface of aquatic plants. Many work have been done on the reproduction and biochemical studies of some fresh water snail such as *Lymnaea acuminate*, Indoplanosbis exustus. S.kumar, D.K.Singh and V.K.Singh (2013)[4] studied the effect of plant molluscides on reproduction of snail *Lymnaea acuminate*. Recently Ethnomedicinal importance of *B . bengalensis* was estimated in terms of analysis of its protein and amino acid composition Debojit.C et *al*. (2015)[5] also studied proximate fatty acid composition of *B .bengalensis*.

The bodies of snails are characterised by rich mucus which covers their surface. Apparently, the mucus may serve in preventing the moisture evaporation, in belong smooth movements (Simkiss and Wilbur, 1977)6 and in protecting the body from mechanical injuries.Mucus has antibacterial properties against many pathogenic bacteria. In addition sum

unknown biochemical function may be involved in the mucus, though nothing has been reported so far with this respect. Animals are fairly resistant to infection by microorganisms. So, present paper studies about antibacterial activity against pathogens.

Materials and method:

- **Collection of sample:**

Snail *B. bengalensis* were purchased from Ranchi market for this part of the study. They were thoroughly cleaned with cleaned napkin to remove all the sand and debris on the shell.

- **Extraction of mucus:**

The mucus were extracted from the snail by removing the shell with a sterile sharp end of metal rod, the mucus aseptically squeezed out from the soft body. It was collected into a beaker(Lawrence B. Etim *et. al* 2016)[7].The extracted mucus was considered 100% concentration and was stored in the refrigerator at 4°c for antibacterial analysis.

- **Antibacterial assay**:

The antibacterial activity of mucus preparation was assayed using the disc diffusion method (DDM). In this method 4mm diameter discs were cut out from Whatman no 1 filter paper and sterilized using autoclave at 121°c for 15 minutes. The sterilized disc was soaked in concentration of 100% (v/v) snail mucus (Lawrence, B. Etim *et al* 2016). Swabs were inoculated on Muller Hinton agar plate for the isolation of bacteria, using the streak plate method. Mucus impregnated discs were thereafter, air dried and placed on the plates already seeded with 1 ml for 18 hour old broth culture at 0.5 Mc Farland Standard (1.5×10^6cfu ml^{-1}) and disc incubated at 37°c for 24 hours. Drug ciprofloxacin used for +ve control.

Result and Discussion:

Table 1: Antibacterial activity of different concentration of mucus (15µl/ disc & 30µl/ disc) against pathogens .

Pathogens	Zone of inhibition (mm) concentration of mucus (µl)/disc	
	15	30
Escherichia coli	12.0±0.0	14.66±.57
Staphylococcus aureus	10.33±0.57	12.66±.57
Proteus vulgaris	10.0±10	12.33±.58

Table 2: Comparison of antibacterial efficacy of snail mucus(30µl/disc) and +ve control using t-test.

Pathogens	Zone of inhibition (mm)	
	Snail mucus (30µl/disc)	Positive Control(30mg/disc)
Escherichia coli	14.66±.57	16.66±.58*
Staphylococcus aureus	12.66±.57	16.66±.57***
Proteus vulgaris	12.33±.58	16.00±1**

*P<0.05 or significant at 5%, **P<0.01 or significant at 1%, ***P<0.001 or significant at 0.1%

Table3 : Comparison of antibacterial efficacy of snail mucus for three pathogens.

Pathogens	Zone of inhibition (mm)
Escherichia coli vs Staphylococcus aureus	14.66±.57* vs12.66±.57
Escherichia coli vs Proteus vulgaris	13.66±.57 *vs12.33±.58
Staphylococcus aureus vs Proteus vulgaris	12.66±.57 vs12.33±.58

The highest antibacterial activity of mucus of *B.bengalensis* against *Escherichia coli* was observed as 13.66±.57 mm zone of inhibition followed by *S. aureus* (12.66±.57 mm) and *Proteus vulgaris* (12.33±.58).

Maximum inhibition zone was found in case of +ve control. Statistical analysis showed that *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris* had lesser antibacterial efficacy than +ve control (P<0.05, P<0.001 and P<0.01) at 5%,0.1%and 1% respectively. Statistical analysis also showed that mucus from *B. bengalensis* had more antibacterial efficacy in *Escherichia coli* than *S.aureus* and *P. vulgaris* (P<0.05). In both bacteria, *S. aureus* and *P. vulgaris* showed no significant difference, both showed same efficacy.

This study revealed that mucus obtained from *B. bengalensis* showed varying levels of antibacterial activity on the three test organism used

(*Escherichia coli, S.aureus* and *Proteus vulgaris*). In table 1 the mucus also showed an increase in antibacterial activity with increase in concentration..

Similar result was reported by Chellaram *et.al*[8] in case of *Trochus radiates* gastropod, maximum antibacterial activities were reported against *S. aureus* and *E. coli*. Lawrence B *et .al* also reported that snail mucus contained antibacterial properties obtained from *Archachatina. marginata. saturalis, Archachatina. marginata ovum* and *Achatina. fulica*. Result revealed that *Staphylococcus sp* was more susceptible to mucus secretion from *A. marginata* (17.4±1.20mm), which corroborate the present finding in case of *B. bengalensis* but in case of *A. marginata* ovum inhibition zone was (15.6±1.44mm) and in *A. fulica* (15.4±2.04mm) indicating that in these cases the antibacterial efficacy is nearly same as *B. bengalensis*.

According to Anand P T *et. al* [9], ethanol extract of gastropods, *B. spirata* and *Turobo. brunneus* also showed antibacterial activity against *K. pneumoniae, P. vulgare's and S. typhi.*

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