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Freshwater edible snail *Bellamya bengalensis* as a source of antibacterial agent against clinical pathogens.

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Abstract- Snails have been used both as a food and as a treatment for a variety of medicinal conditions. In this study, *Bellamya bengalensis* muscle extract was evaluated for antibacterial activity. The extracted muscle was estimated antibacterial properties by using Disc diffusion method. Snail muscle extract was evaluated for antimicrobial activity against two pathogenic bacterial (*Salmonella typhi* and *Micrococcus luteus*). This muscle extract has ability of inhibiting the development bacterial growth. Inhibition zone was found 19.66±0.72 and 20.66±1.15 in *Salmonella typhi* and *Micrococcus luteus* respectively. This research shows that the snail *Bellamya bengalensis* could be used as an antibiotic.

Key words: Antibacterial activity, *Bellamya bengalensis*, Disc diffusion method, *Salmonella typhi, Micrococcus luteus*.

INTRODUCTION

Snail has a calcareous shell to contain the animal body.[1] Snails are found in every continent on Earth, in freshwater, marine water, and terrestrial habitats.[2] These snails were characterized by their nutritional efficacies and biomedical properties, and the present research also showed some of these important properties and usages. Marine organisms are considered as a potential source for naturally bioactive compounds. In which, mollusk- the largest group possesses antioxidant, anti-inflammatory, cytotoxic, antibacterial, antifungal, and anticancer properties.

Antimicrobial agents are used in treating bacterial, parasitic, fungal and viral infections while antibiotics are types used in treating and preventing pathogenic bacteria. These agents may either destroy or prevent the growth of bacteria. Those which kill the bacteria are called bactericidal while bacteriostatic types prevent growth while before surgical cases, some antibiotics can be given as a prophylactic to prevent especially bacterial infection. Various diseases which were fatal prior to the development of antibiotics have been effectively treated using these antibacterial agents. Some of the antibiotics have been used as cancer therapy.[3]

At the right dosage, the use of antibiotics has a significant number of benefits to humans. These agents have facilitated the reduction of deaths every year caused by several infectious diseases. Since antibiotics were first developed, clinicians were excited about their success in eliminating pathogens, such that in the 1970, a US Surgeon General made a statement that the agents have won the war against diseases.[4] However, these antibiotics may have side effects if they are overly used.

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MATERIALS & METHODS

Collection of samples

Snail, *Bellamya bengalensis* were collected from nearest ponds and also purchased from local market for this research and kept into separate aquarium for 7-15 days for acclimatization, water was changed on alternate day.

Extraction of the animal tissues.[5]

The extraction of muscle from the snail was done by removing the shells. 50gm fresh muscle was soaked in ethanol and maintained for 3 days. The extracts were filtered through Whatman no.1 filter paper and concentrated by evaporation using hot plate at 30°C to obtain a dark brown gummy mass. The resultant residues were stored at 4°C for further analysis.

Antibacterial effect of muscle extract of snail *B. bengalensis* on human pathogens.

Collection of drugs and organism : The Ciprofloxacin drug was collected from RIMS. Pathogens were collected from the patient sample of RIMS from Microbiology department, Ranchi.

Strains of 2 bacterial cultures were used in this study.

Microbial pathogens used: *Salmonella typhi* (Gram-negative, rod shaped), *Micrococcus luteus* (Gram-positive, spherical shaped).

Preparation of disc : 6 mm disc was prepared from Whatman no. 1 filter paper and sterilized for 15-20 minutes. Sterilized disc was soaked in muscle extract for antibacterial testing.

Preparation of inoculums : Identical colonies were isolated and sub-cultured in 2ml of nutrient broth for 4 hours at 37°C. Standardization of the inoculums was made by preparing a 0.5 Mc. Farland standard and compared with the turbidity of the inoculums.

Antibacterial assay : Antibacterial activity was carried out by using standard disc diffusion method (DDM).[6] The test cultures (bacterial 1.5×10^6 cfu/ml) were swabbed on top of the solidified media and dry it for 10 minutes. Drug ciprofloxacin used for +ve control. The human bacteria were maintained on nutrient agar plates. Extract of snail muscle was impregnated on to 6mm sterile discs of Whatman no. 1 filter paper. Impregnated discs were placed on nutrient agar plates seeded with isolated microorganisms and incubated at 37°C for 24 hours. The susceptibility of the test organisms were determined by measuring the radius of the inhibition zone around each disc.

Statistical Analysis : The differences obtained in measurement of inhibition zone were statistically analyzed using student's t-test.

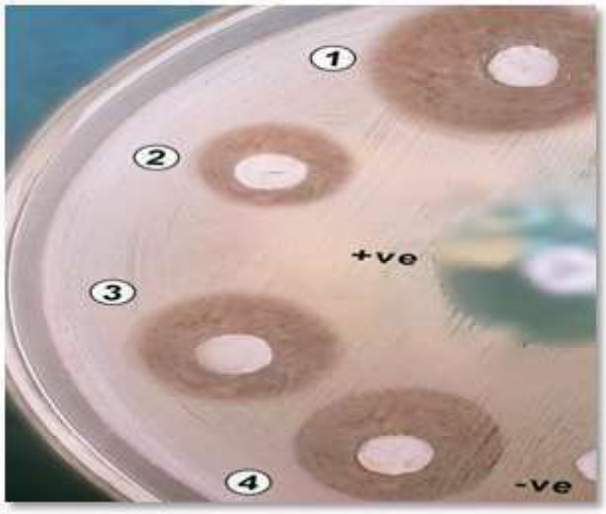

Fig. 1- Antibacterial activity of snail's muscle against *Salmonella typhi* (100mg/disc).
Abbreviations : 1, 2 and 3 - Zone of inhibition of snail's muscle. +ve - Zone of inhibition of Drug ciprofloxacin as positive control

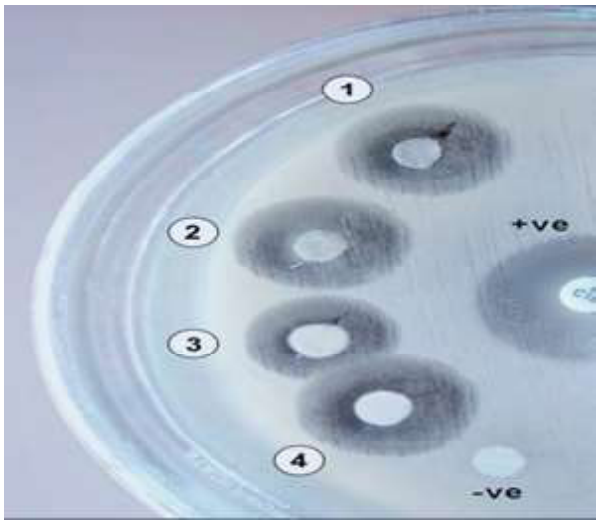

Fig. 2- Antibacterial activity of snail's muscle against *Micrococcus luteus* (100mg/disc).
Abbreviations : 1, 2 and 3 - Zone of inhibition of snail's muscle. +ve - Zone of inhibition of Drug ciprofloxacin as positive control

Table 1: Comparison of antibacterial efficacy of snail's muscle (100mg/disc) and +ve control using t-test.

Pathogens	Zone of inhibition (mm)	
	Snail muscle (100mg/disc)	Positive Control (100mg/disc)
Salmonella typhi	19.66±0.72	2.33±0.57**
Micrococcus luteus	20.66±1.15	20.00±00

**P<0.01 or significant at 1%.

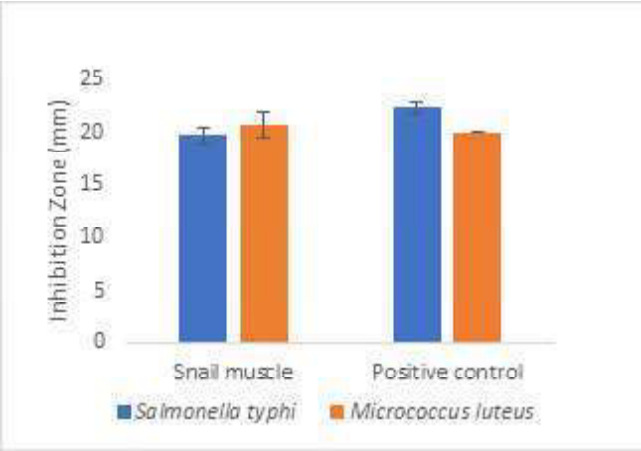

Fig. 3- Antibacterial activity of snail's muscle extract (100mg/disc) on human pathogens (*Salmonella typhi* and *Micrococcus luteus*).

Snail's muscle at concentration of 100mg/disc showed antibacterial activity against *Salmonella typhi* and *Micrococcus luteus* with inhibition zone of 19.66±0.72mm and 20.66±1.15mm respectively (table-1, fig-1, 2 and 3). The highest antibacterial activity of muscle of *B. bengalensis* against *Micrococcus luteus* was observed as 20.66±1.15mm zone of inhibition followed by *Salmonella typhi* i.e., 19.66±0.72mm. Maximum inhibition zone was found in case of +ve control. Statistical analysis showed that *Salmonella typhi* had lesser antibacterial efficacy than +ve control and in *Micrococcus luteus* showed antibacterial efficacy same as positive control.

DISCUSSION

In present study, sensitivity test of muscle extract from *B.bengalensis* snail showed antimicrobial activity at low concentration against two tested pathogenic bacteria. The extract from *B.bengalensis* has ability to completely inhibited the growth of pathogenic bacterial. The gastropods showed antibacterial activity against *S. typhi* and this is reported by Rajaganapathi *et al.* (2000)[7]. The crude extract of Caribbean Sea invertebrates and bactericidal proteins from the Littoral Mollusk *Cenchritis muricatus* showed the antibacterial activity which is evaluated by Lopez *et al.* (2012)[8]. The antibacterial activity of marine gastropod *Hemifusus pugilinus* also reported by Sugesh *et al.* (2013)[9]. The ethanol extract of the freshwater gastropod *P. virens* showed antibacterial activity and reported by Gayathri. M. & Sanjeevi S. B. (2014)[10]. The molluscan garden snail *Helix lucorum* hemolymph peptides showed antimicrobial activity and inhibit the growth of *S. epidermidis*, *E. coli* and *S.aureus,* and which was investigated by Dolashka *et al.* (2015)[11].

CONCLUSION

Gastropods snail proved that it could be source of antibacterial agents. This research is helpful for those people which believe in natural product and it has no side effects on health.

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