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Antibacterial Activities of Coelomic Fluid of Local Earthworms against Disease Causing Microorganisms

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The antibacterial properties of coelomic fluid of local earthworms (*Pontoscolex corethrurus, Megascolex konkanensis, Drawida ghatensis*) against selected pathogens like *Vibrio cholera, Vibrio parahaemoliticus, Staphylococcus aureus, Salmonella typhi and Escherichia coli*. The study was conducted in School of Environmental Science and College of Veterinary and Animal Science in May 2017 to November 2017. The earthworm was collected on the basis of hand sorting method and Coelomic fluid was collected due to cold shock drips. Antimicrobial activity of coelomic fluid of earthworms was assessed by agar well diffusion method. The result revealed that the maximum inhibition zone of 18 mm and 16 mm showed by coelomic fluid of *P. corethrurus* and *M. konkanensis* against *S. aureus*. The *Drawida ghatensis* shows least antibacterial activities against

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selected pathogens. The *staphylococcus aureus* shows highest inhibition zone against coelomic fluid of all the selected earthworms and least result showing in bacterial isolate *vibrio parahaemoliticus*. From the study coelomic fluid activity is good for pathogen like salmonella typhi and *E. coli* and have medicinal values.

Keywords: Antibacterial activity; coelomic fluid; Pontoscolex corethrurus; Megascolex konkanensis; Drawida ghatensis.

ABBREVIATIONS

P. corethrurus :Pontoscolex corethrurus
M. konkanensis :Megascolex konkenensis
D. ghatensis : Drawida ghatensis
V. cholera :Vibrio cholera
V. parahaemoliticus :Vibrio parahaemoliticus
S. aures :Staphylococcus aureus
S. typhi :Salmonella typhi
E. coli :Escherichia coli

1. INTRODUCTION

The Earthworm (Kingdom: Animalia, Phylum: Annelida, Class: Oligochaeta) are familiar to almost everyone [1]. The role of some species in organic matter decomposition and mineral cycling may be important [2] and a great deal has been written concerning earthworm farming [3]. The activities of earthworms that affect the soil involve the ingestion of soil and the mixing of the main soil ingredients of clay, lime, and humus; the construction of burrows that enhance aeration, drainage, and root penetration. The influence of earthworms on the translocation of soil material may be quite considerable [4]. The body of earthworm is divided into a serious of uniformly placed segments. This nature of division of the body both externally and internally has enabled animal to have flexibility and for the initiation of development of good musculature. Earthworms are the first group of animals to have complete digestive system, closed circulatory system with haemoglobin in the plasma as carrier of oxygen and carbondioxide [5].

The pathogens are firstly bacteria living in water or soil that are ingested during feeding or introduced into the body following injury. During the course of evolution, earthworms have developed defense strategies against these living pathogens. Earthworms lack true antibodies and hence an adaptive immune response and instead have efficient innate immunity system to defend themselves against invading foreign materials. In living organisms, peptides are an important defense component, many peptides were found in various living organisms.

The earthworms are coelomate animals and filled with coelomic fluid – a milky alkaline liquid that helps the worm in locomotion nutrition, excretion, detoxification of tissues, heavy metal accumulation and protects internal organs from external jerks, destroys bacterial attack, prevents desiccation, promotes cutaneous respiration and internal acclimation [6,7]. The coelomic fluid of earthworms and their body extracts were known to have antimicrobial and many medicinal properties since 1340. Studies done by many workers have strongly pointed out that coelomic fluid of earthworm like *Eisenia foetida, Eudrilus euginae, Polyphertima elongate, Perionyx excavates, Lampito maurtii, L. rubellus and Perionyx sansibaricus* have medicinal and antibacterial properties. Earthworm has been recognized in prenatal medicine and anti-inflammatory, analgesic and antipyretic agent.

Except for congenital diseases, all other diseases are caused by certain microorganisms. Such microbes are called pathogens. They may affect or damage the whole body system or some specific tissues or organs. Some human diseases caused by pathogens are polio, cholera, mumps, rabies, malaria etc. Plants and animals are also victims of these microbial effects. Some microorganisms need vectors or agents for their transmission. Malaria is an example of the harmful microorganism [8].

It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious*, S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. Typhoid fever, also known simply as typhoid, is a bacterial infection due to *Salmonella typhi* that causes symptoms. Symptoms may vary from mild to severe and usually begin six to thirty days after exposure. Often there is a gradual onset of a high fever over several days [9].

Weakness, abdominal pain, constipation, and headaches also commonly occur. Cholera is an acute, diarrheal illness caused by infection of the intestine with the bacterium *Vibrio cholerae.*

Vibrio parahaemolyticus is a self-limiting, enterotoxic bacterium, typically causing acute gastroenteritis in humans. More severe cases of infection can occur in immune-compromised individuals, which can lead to septicemia and death, although this is very rare. Moderate to severe skin infections can also result from open wound exposure to *V. parahaemolyticus* in warm seawater, although this occurs less frequently than illness following ingestion of the organism [10]. *E. coli* refers to a wide range of bacteria that can cause various diseases, including pneumonia, urinary tract infections, and diarrhea. Most strains of *E. coli* are harmless to humans. Some strains of *E. coli* infection can include nausea, vomiting, and fever.

- To characterize the antibacterial activities of coelomic fluid of local earthworms against disease causing microorganisms.
- To characterize the antibacterial activity of pathogenic bacteria using well diffusion method.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Earthworms

Hand sorting method was used for collecting earthworms. This method is widely used for sampling earthworms in India. The quadrate is provided on 20*20*30 cm2 and are gently broken and the worms are hand sorted [10,11]. The earthworm samples were collected from the field were pesticides cannot be used. The collected earthworms were identified with the help of standard manual and experts. The study mainly focused on three species of earthworms both exotic and native such as *Pontoscolex corethrurus* [12], *Megascolex konkanensis* [13] and *Drawida ghatensis* [14].

2.2 Microorganisms Used

Test organisms were collected from the Environmental Microbiology Lab, School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala. These include the standard cultures of *Vibrio cholera, Vibrio parahaemoliticus, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli.*

2.3 Coelomic Fluid Collection Method

The selected local earthworms were washed in distilled water and they were placed on ordinary wet filter paper in plastic tough which is covered by aluminum foil with fine pin holes. After 48 hrs, the gut was cleared of organic matter as they feed on filter paper. Coelomic fluid was collected by placing the earthworms in petri plates held in a slanting position in the palm. Their body surface was rubbed with wet finger and later with ice cubes taken in a beaker. The coelomic fluid released due to cold shock drips and gets collected at the lower side of the Petriplate. The released fluid from their body was collected by Pasteur pipette [15].

2.4 Antimicrobial Activity (Well Diffusion Method)

The young culture of selected pathogens *Vibrio cholera (1), Vibrio parahaemoliticus(2), Staphylococcus aureus(3), Salmonella typhi(4)* and *E. coli(5)* were prepared in nutrient

Pontoscolex corethrurus *Megascolex konkanensis* *Drawida ghatensis*

Fig. 1. Earthworms selected for the study

broth(1.3 gm in 100 ml and inoculated in 10 ml) and lawn culture of different pathogens were prepared by swabbing young culture (16-18 hrs) in Muller Hinton agar and waited for 15 minutes to absorb the culture to the medium. The bacterial cultures inoculated in Nutrient Agar broth were incubated at 37°C for 18 hr. The suspension were checked to provide approximately 10^5 cfu/ml. Agar wells (3mm) in diameter were punched in the plates using a sterile gel puncture. 30 µL of coelomic fluid were pipetted into the well and plates were incubated for 24 hrs in an incubator. Zone of inhibition around the wells were recorded in mm.

2.5 Statistical Analysis

The statistical analysis was performed using GraphPad Prism version 5 for Windows, GraphPad Software, California, USA [16]. The antibacterial properties of earthworms against selected bacterial strains, one-way ANOVA was used. The mean score of three species of earthworm indicate that the significant difference in the antibacterial properties.

3. RESULTS AND DISCUSSION

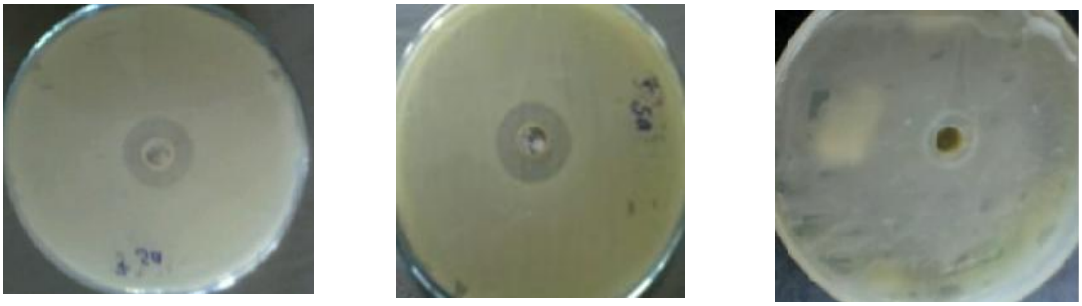
The coelomic fluid of selected earthworms *Pontoscolex corethrurus, Megascolex konkanensis* and *Drawida ghatensis* was collected by cold shock method were tested for antibacterial activity against five pathogenic bacteria using well diffusion method. The antibacterial property of coelomic fluid against selected five bacterial strains was found that *M. konkanensis* shows high zone of inhibition for *S.aureus (18mm)* Fig. 2(1) compare with all the three earthworms. The *P. corethrurus* shows high antibacterial activity against S*. aureus* then *V.cholera* (16mm and 14mm) and the least activity shows in *V. parahaemoliticus* (8mm) (Table 1 and Fig.4). In *P. corethrurus* no activity shown in *S. typhi. M. konkanensis* shows higher zone of inhibition against *S. aureus*, follows *E. coli, then S.typhi* (18 mm,15 mm, 14 mm) (Table 1 and Fig. 5) no ativity shown in *V. parahaemoliticus. D. ghatensis* shows higher zone of inhibition in *S. typhi,* (10mm) follows *S.aureus* (9mm) and *no* activity shown in both *E. coli, V. cholera* and *V. parahaemoliticus* (Table 1 and Fig. 6). *A* maximum inhibition zone of 18 mm and 16 mm [Fig. 2(2)] showed by coelomic fluid of *P. corethrurus and M. konkanensis against S. aureus.*

The extraction of coelomic fluid from earthworms, cold shock method is a novel method as it won't require any apparatus and more importantly used earthworms are unharmed and show normal activities even after collection of coelomic fluid several times. The coelomic fluid of selected earthworms shows good antibacterial properties and has good medicinal value. The antibacterial property of coelomic fluid in the *Megascolex konkenensis* shows high medicinal values against *Staphylococcus aureus* and *E. coli*. The selected pathogens are common disease causing bacteria. The coelomic fluid contains several bioactive compounds such as proteins, exhibits a variety of biological functions such as antibacterial, anticancer, haemolytic, cytotoxic, hemagglutinating and proteolytic activities etc [17].

Recently, another two antimicrobial peptides (PP1 and OEP3121) have been identified from earthworms of *Pheretima tschiliensis* and *E. foetida*, respectively [18]. The antimicrobial peptide, lumbricin-PG was identified from skin secretions of the earthworm, *Pheretima guillelmi* [19]. [20] found that earthworm coelomic fluid contains biologically active molecules and leukocytes that participate in phagocytosis, encapsulation and killing of HeLa, HEp-2, PC-12 and PA317 cells *in vitro* [21]. Earthworms synthesize and secrete several effective modulators of innate immune responses such as antibacterial molecules, cytotoxic proteins and cytokines [9]. The gut extracts of earthworms

Table 1. Zone of inhibition of earthworms against selected bacterial strain

Bacterial isolates	Zone of inhibition(mm)		
	P. corethrurus	***M. konkanensis***	***D. ghatensis***
V. cholera	14	10	0
V. parahaemoliticus	8	0	0
S. aureus	16	18	9
S. typhi	0	14	10
E. coli	11	15	0



(1) (2) (3)

Fig. 2. Sensitivity of high zone of inhibition *M. konkanensis* against *S. aureus (1) P. corethrurus* against *S. aureus (2)* and *D. ghatensis* against *S. typhi(3)*

(1) (2) (3)

Fig. 3. Sensitivity of least zone of inhibition *M. konkanensis* against *V. cholera (1) P. corethrurus* against *V. parahaemoliticus (2)* and *D. ghatensis* against *S. aureus(3)*

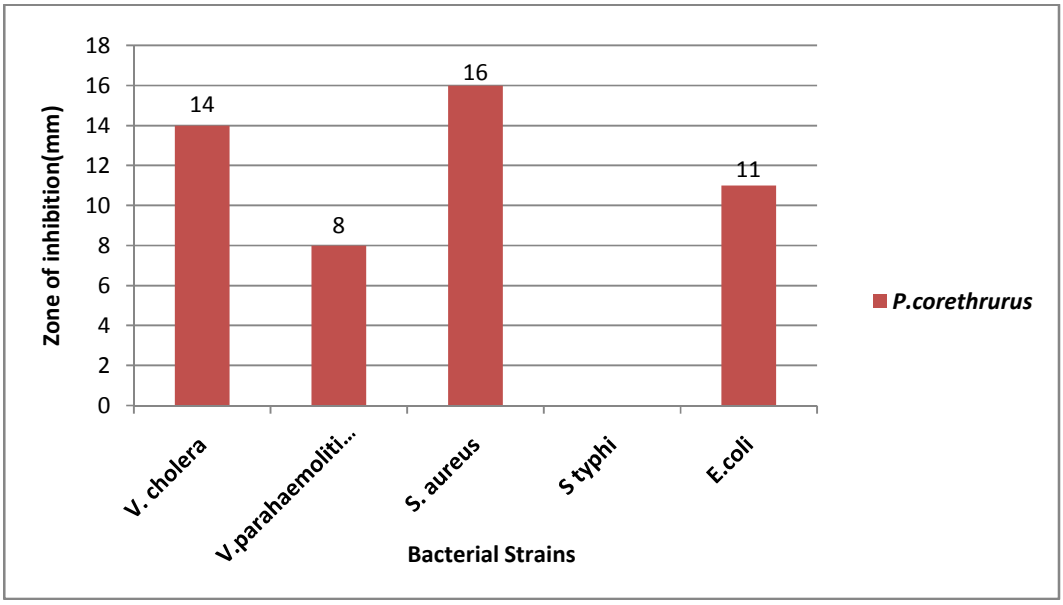


Fig. 4. Antibacterial activity of *P. corethrurus* against selected bacterial strains

have antibacterial and antifungal activity [17]. The new bacterial strain with antimycobacterial activity has been isolated from the midgut of *Dendrobaena veneta* [22].

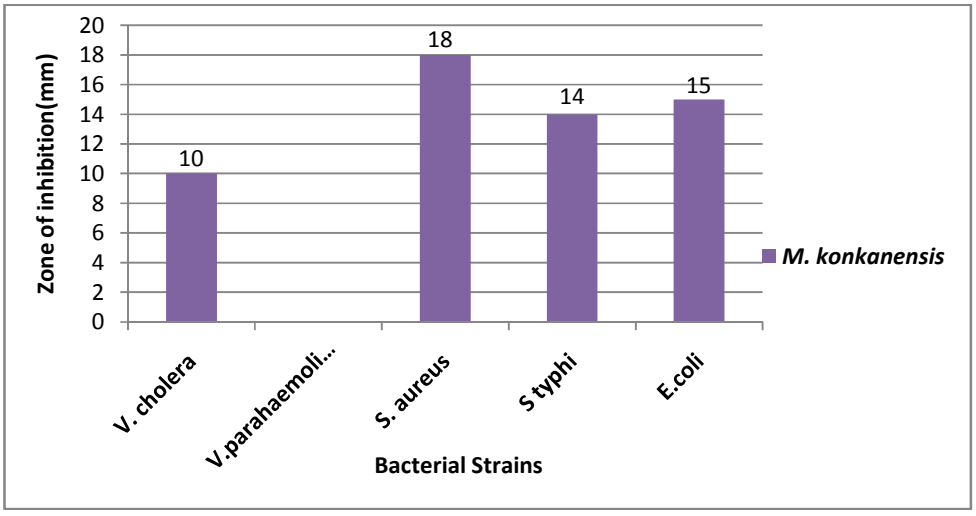


Fig. 5. Antibacterial activity of *M. konkanensis* against selected bacterial strains

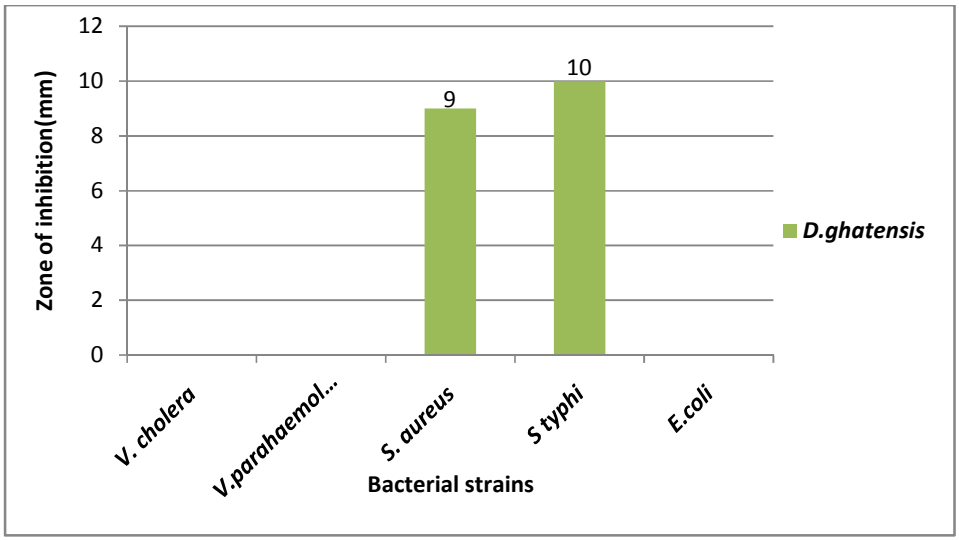


Fig. 6. Antibacterial activity of *D. ghatensis* against selected bacterial strains

4. CONCLUSION

The experimental investigation revealed that Antibacterial Activities of Coelomic Fluid of Local Earthworms against Disease Causing Microorganisms. The coelomic fluid of *P. corethrurus, M. konkanensis, D.ghatensis* shows antibacterial properties against V. cholera, *V. parahaemoliticus, S. aureus, S. typhi and E. coli*. The *M. konkanensis* shows high antibacterial activity for these two bacterial strains. *D. ghatensis* shows least antibacterial activity. So this work suggests some of the coelomic fluid components might be useful for pharmaceutical applications and for purifying the biomolecules to introduce in the field of pharmaceuticals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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