



Antibacterial and Antifungal Potentialities of Earthworm *Eudrilus eugeniae* Paste and Coelomic Fluid

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Authors' contributions

This work was carried out in collaboration between all authors. Author KCS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors APT and KCR managed the analyses of the study. Author KCR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A study was carried out on the antibacterial and antifungal properties of earthworms *Eudrilus eugeniae* paste and coelomic fluid against selected pathogens like *Vibrio cholera*, *Vibrio parahaemolyticus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. The study was conducted on School of Environmental Sciences, Kottayam. The coelomic fluid and paste of earthworms was screened against some human pathogenic bacteria including six bacterial strains and three fungal strains. The earthworm was collected by handsorting method. The earthworm paste were prepared and coelomic fluid was collected by heat and cold shock method. The antimicrobial activity was done through the

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agar well diffusion method. The result revealed that the antibacterial activity in earthworm paste shows that *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* shows 16mm, 8mm, 18mm diameter. The other bacterial isolates shows no zone of inhibition. The zone of inhibition of coelomic fluid against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* shows 10mm, 14mm, 16mm simultaneously. The *Vibrio parahaemolyticus* shows slight changes in the zone of inhibition area. *Vibrio cholera* and *Staphylococcus typhi* shows no activity against paste and coelomic fluid of earthworm. The antifungal activity of earthworm *Eudrilus eugeniae* against fungal strains like *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. The coelomic fluid of *Candida albicans* 18mm shows high zone of inhibition compared to *Aspergillus niger* 16mm. The earthworm paste of *Eudrilus eugeniae* shows antifungal property against *Aspergillus niger* (14 mm). *Aspergillus flavus* shows no activity against paste and coelomic fluid of earthworm. The present work concludes that the *Eudrilus eugeniae* paste and coelomic fluid shows antibacterial and antifungal properties against selected bacterial and fungal isolates and suggests some of the coelomic fluid and paste components might be useful for pharmaceutical applications.

Keywords: Antibacterial; antifungal; *Eudrilus eugeniae*; coelomic fluid.

1. INTRODUCTION

The earthworm is a tube-shaped, segmented worm found in the phylum Annelida. Earthworms are commonly found living in soil, feeding on live and dead organic matter [1]. An earthworm's digestive system runs through the length of its body [2]. It conducts respiration through its skin. It has a double transport system composed of coelomic fluid that moves within the fluid-filled coelom. While earthworms, the largest of the Oligochaeta, have medicinal properties, they are also related to various other species, such as leeches, that have been shown to exhibit therapeutic benefit [3]. Specifically, earthworms have been vital to investigations of developmental organizations of the nervous, immune, and endocrine systems. Not only are earthworms important in understanding biological processes of development [4] but also their well-known significance to ecosystems, specifically their ability to improve soil fertility. Earthworms have been utilized as a form of nutrition as well as treatment for specific conditions as traditional forms of medicine such as those in Asia [5]. The field of examining the potential health benefits of consuming earthworms owes its increasing interest to bioprospecting [6]. Researchers have discovered that in addition to ecological benefit, specifically soil preservation, the long neglected earthworm innate immune system holds the key to these observed medicinal qualities [7].

The study aims to find the antibacterial and antifungal properties of earthworms *Eudrilus eugeniae* paste and coelomic fluid against selected pathogens. The bacterial strains used were *Vibrio cholera*, *Vibrio parahaemolyticus*,

Bacillus subtilis, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and fungal strains used were *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. The antibacterial and antifungal studies conducted using well diffusion method.

2. MATERIALS AND METHODS

2.1 Collection of Earthworm

Hand sorting method was used for collecting earthworms. This method is widely used for sampling earthworms in India [8,9]. The quadrat is provided on 20*20*30cm² and are gently broken and the worms are hand sorted [7]. The earthworm samples were collected from the field where pesticides cannot be used. The collected earthworms were identified with the help of standard manual and experts. The study mainly focused on earthworm *Eudrilus eugeniae*.



Fig. 1. *Eudrilus eugeniae*

Scientific Classification (<i>Eudrilus eugeniae</i>)	
Kingdom	Animalia
Phylum	Annelida
Class	Clitellata
Subclass	Oligochaeta
Order	Haplotaxida
Family	Eudrilidae
Genus	<i>Eudrilus</i>
Species	<i>eugeniae</i>

2.2 Preparation of Earthworm Paste

The earthworm were washed with running tap water and then fed with wet blotting paper for 18 to 20 hrs to clear their gut. The gut cleared worms were again washed with distilled water. The worms were kept in plastic troughs, covered tightly with polythene cover, and exposed to sunlight for 3 days to kill them. Mucus and coelomic fluid that oozed out digested the dead worms forming a brown colored earthworm paste(EP) [10].The earthworm paste were filtered and the filtrates obtained were condensed in water-bath at 35°C.



Fig. 2. Coelomic fluid

2.3 Coelomic Fluid Collection Method

The species of 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. They were thoroughly washed with distilled water and then placed in glass funnel, warm water (40 – 50°C) in a glass beaker (10 ml) was used to give heat shock and in a similar way ice cubes were used to give cold shock. The treatment was alternately at a gap of three minutes to overcome the shock effect. This

caused agitation for the coelomic fluid to ooze out from dorsal pores and mouth of earthworms. The fluid released was collected into pipette. Earthworms were released released back to the culture tubs after collection of coelomic fluid.

2.4 Test Microorganisms for Antimicrobial Studies

For the antimicrobial screening, six species of bacterial isolate and three species of fungal isolates were selected. The bacterial strains were obtained from School of Environmental Sciences, Kottayam and fungal strains were obtained from Tropical Institute of Ecological Sciences, Kottayam. *Vibrio parahaemoliticus* *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Vibrio cholera*, strains were used. *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* fungi were used as test organisms. The bacterial cultures were used for antimicrobial test maintained on nutrient agar slant and the fungal strains were maintained on Sabouraud dextrose agar slant at 4°C. The fresh bacterial cultures were obtained by growing the test organisms at 37°C, for 24 h while fungi were grown at 28°C for 48 h.

2.5 Antibacterial Potentialities Using Well Diffusion Method

2.5.1 Earthworm paste and coelomic fluid

The young culture of selected pathogens *Vibrio cholera* (A), *Vibrio parahaemoliticus*(B), *Bacillus subtilis*(C), *Staphylococcus aureus*(D), *Salmonella typhi*(E) and *E.coli*(F) were prepared in nutrient broth(1.3 gm in 100 ml and inoculated in 10ml) 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. Agar wells (3 mm) in diameter were punched in the plates using a sterile gel puncture. 30 µL of earthworm paste solution and coelomic fluid were pipetted into the well and plates were incubated for 24 hrs in an incubator [11]. Zone of inhibition around the wells were recorded in mm.

2.6 Antifungal Potentialities Using Well Diffusion Method

2.6.1 Preparation of inoculums

Suspension of fungus was prepared as per Mac-ferland Nephelometer standard. A 24 hr old

culture was used for the preparation of fungus suspension. A suspension of fungus was made in distilled water and the turbidity was adjusted such that it contained approximately 1.5×10^8 cells/ml. The inoculum was prepared by mixing dextrose, peptose and distilled water.

2.6.2 Earthworm paste and coelomic fluid

The young cultures of selected pathogens (*Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*) were prepared in broth and lawn culture of different pathogens were made by swabbing young culture in 5-6 days old in potato dextrose agar and sabourauds dextrose agar and waited for 15 minutes to absorb the culture to the medium. Agar wells (3 mm) in diameter were punched in the plates using a sterile gel puncture .30 μ L of a five day old culture of all the selected fungal strains in appropriate broth and paste and coelomic fluid were pipetted into the well and plates and incubated for 4-5 days at room temperature. Zone of inhibition around the wells were recorded in mm.

3. RESULTS AND DISCUSSION

Earthworm paste and coelomic fluid prepared from *Eudrilus eugeniae* was tested for antibacterial and antifungal activities. For antimicrobial assay, six strains of bacteria *Vibrio parahaemolyticus*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*,

Salmonella typhi, *Vibrio cholera* and three strains of fungus.

Candida albicans, *Aspergillus niger*, *Aspergillus flavus* were used for antibacterial and antifungal activity. Of the six bacteria tested, the growth of bacteria was well inhibited by earthworm paste and coelomic fluid.

3.1 Antibacterial Activity of Earthworm Paste and Coelomic Fluid

The antibacterial activity was determined by measuring the diameter of zone of inhibition recorded in mm. The antibacterial activity of earthworm *Eudrilus eugeniae* against bacterial strains like *Vibrio parahaemolyticus*, *Vibrio cholera*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi*. The antibacterial activity in earthworm paste shows that *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* shows 16mm, 8mm, 18mm diameter (Table.1). The zone of inhibition is high against *staphylococcus aureus*(Fig. 3). The other bacterial isolates shows no zone of inhibition. The zone of inhibition of coelomic fluid against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* shows 10 mm, 14 mm, 16 mm simultaneously shown in (Table 1 and Fig. 4). The *V. parahaemolyticus* shows slight changes in the zone of inhibition area (Fig. 5). *Vibrio cholera* and *Staphylococcus typhi* shows no activity against paste and coelomic fluid of earthworm.

Table 1. Antibacterial sensitivity of earthworm paste and coelomic fluid

Bacterial strains	Zone of inhibition earthworm pastes (mm)	Zone of inhibition coelomic fluid of earthworm (mm)
<i>Vibrio parahaemolyticus</i> ,	0	Slight change in the zonal area
<i>Vibrio cholera</i>	0	0
<i>Escherichia coli</i>	16	10
<i>Bacillus subtilis</i>	8	14
<i>Staphylococcus aureus</i>	18	16
<i>Salmonella typhi</i>	0	0

Table 2. Antifungal sensitivity of earthworm paste and coelomic fluid

Fungal strains	Zone of inhibition earthworm pastes (mm)	Zone of inhibition coelomic fluid of earthworm(mm)
<i>Candida albicans</i>	0	18
<i>Aspergillus niger</i>	14	16
<i>Aspergillus flavus</i>	0	0

3.2 Antifungal Activity of Earthworm Paste and Coelomic Fluid

The antifungal activity was determined by measuring the diameter of zone of inhibition recorded in mm. The antifungal activity of earthworm *Eudrilus eugeniae* against fungal strains like *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. The coelomic fluid of *Candida albicans* 18mm shows high zone of inhibition (Fig. 6) compared to *Aspergillus niger* 16mm (Fig. 6). The earthworm paste of *Eudrilus eugeniae* shows antifungal property against *Aspergillus niger* (14mm)(Fig. 6). *Aspergillus flavus* shows no activity against paste and coelomic fluid of earthworm. The coelomic fluid of earthworm show high resistance against *Aspergillus niger*.

Earthworms contain a lot of proteins that have important roles in biological activity in the body. Here are the benefits of earthworms that are good for our bodies[12]. Padmashree Kulkarni and Geeta Kaicker studied antibacterial property of coelomic fluid of *Eudrilus eugeniae* against *Vibro alginolytions* and *Staphylooccus homimis*. Therefore this clearly indicates that the CF of

Eudrilus eugeniae is the source to look for the biomolecule that can be used for suppressing the activity of pathogens [13]. According to Aydogduan Cotuk *D.veneta's* coelomic fluid in different dilution is ineffective against *A.calcoacetius*. This it indicates that all worms producing their earthworm coelomic fluid and paste are not effective against all bacteria and fungus.

Various solvent extracts of an earthworm, *Eudrilus eugeniae* were prepared and antimicrobial activity of these extracts were determined using well diffusion method against *Staphylococcus aureus* and *Streptococcus pyogens* [14]. Antibacterial & antifungal activities of the whole tissue extract of earthworm, *Eudrilus eugeniae* was tested using well diffusion method [15]. Various solvent extracts of dried earthworm powder (*Lampito mauritii*) were prepared and subjected to preliminary screening for antimicrobial activity [16]. Studies on paste prepared from earthworm, *Eudrilus eugeniae* was tested for antibacterial, antifungal activities. For the antimicrobial screening, four species of bacterial isolate and two species of fungal isolates were selected [17].



Fig. 3. Sensitivity of *Eudrilus eugeniae* paste against *Escherichia coli*(1), *Bacillus subtilis*(2) and *Staphylococcus aureus*(3)



Fig. 4. Sensitivity of *Eudrilus eugeniae* coelomic fluid against *Staphylococcus aureus*(1), *Escherichia coli*(2) and *Bacillus subtilis*(3)



Fig. 5. Sensitivity of *Eudrilus eugeniae* coelomic fluid against *vibrio parahaemolyticus*



Fig. 6. Sensitivity of *Eudrilus eugeniae* coelomic fluid against *Candida albicans* (1) and *Aspergillus niger*(2) and paste against *Aspergillus niger*(3)

4. CONCLUSION

From the present work conclude that the *Eudrilus eugeniae* paste and coelomic fluid shows antibacterial and antifungal properties against selected bacterial and fungal isolates . So this work suggest some of the coelomic fluid and pastes components might be useful for pharmaceutical applications and it can have way for purifying the biomolecules to introduce in the field of pharmaceuticals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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