



Coelomic Fluid of Earthworm In Suppression of Bacterial Pathogens

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ABSTRACT

Since earthworms live in hostile conditions, they have well-developed defense mechanisms to fight against the invading microbes. In the present study antibacterial activity of the coelomic fluid of earthworm, *Eudrilus eugeniae* (EE) was tested against selected Gram positive bacterial pathogens namely, *Staphylococcus aureus* and *Bacillus subtilis* procured from MTCC, Chandigarh, India. Two different screening techniques were followed under laboratory conditions to look for efficiency of coelomic fluid in suppressing growth of the pathogens. Coelomic fluid (CF) was collected from earthworm body cavity by cold shock method. *In vitro* antibacterial assay was done by agar dilution and disc diffusion techniques. The results indicate that *S. aureus* is the most sensitive organism between the two tested pathogens. Tetracyclin and Streptomycin standard discs were used for positive control. The level of suppression by CF was almost on par with that of tetracyclin. This preliminary study is an indication that there is a possibility for identifying and using the antibacterial factor from CF of earthworm to suppress these pathogens in place of prevailing antibiotics in the market.

Keywords: Coelomic fluid, Gram positive bacteria, Tetracyclin, Streptomycin, Antibacterial factor

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INTRODUCTION

Staphylococcus aureus continues to cause nosocomial and community associated skin infections. The incidences of MRSA (Methicillin resistant *Staphylococcus aureus*) have raised a cause of concern. Its incidence varies from 25% in the West to 50% in India. *S. aureus* is a Gram positive organism of public health importance. It has been chosen to study resistance patterns¹. The therapeutic properties of earthworms have been identified and have been used in traditional Chinese medicines since a long time ago². Stevenson³ first reported the pharmaceutical value of earthworms. Wound healing capacities of earthworms have been described. Since they live in hostile conditions, they have well-developed defense mechanism to fight against the invading microbes. The bacteriostatic activity of coelomic fluid of earthworms has been recognized. Tumorolytic and fibrinolytic properties are also attributed to the coelomic fluid⁴⁻⁸. Based on the available information on their role in possible pharmaceutical applications, the present investigation was considered to evaluate the antimicrobial activity of CF of earthworm, EE against two Gram positive pathogens *S. aureus* and *B. subtilis*.

MATERIALS AND METHOD

Bacterial cultures:

The lyophilized bacterial strains of *S. aureus* (MTCC 3160) and *B. subtilis* (MTCC 3055) were procured from IMTECH, Chandigarh. The lyophilized cultures were revived by the addition of 0.3 ml to 0.4 ml of sterile nutrient broth. A loopful of the suspension was then streaked on Nutrient agar. The plates were then incubated for 24-48 hours at 37° C. They were revived and maintained on Nutrient agar medium

Collection of CF:

Adult earthworms with clitellum were selected. They were collected from the culture rings maintained at the Institution and were washed with sterile distilled water. They were then fed on wet filter paper for 48 hours to clear their gut from organic waste. The filter paper fed earthworms were then washed and dried by gently pressing on a blotting paper to remove the excess moisture adhered to body surface. Earthworms were then taken in batches of 4-5 and placed in a Petri plate. A beaker with ice cubes was run over the earthworms to give cold shocks⁹. CF oozed out from the dorsal pores and mouth of the earthworms. It was collected using sterile dropper into a sterile vial. After collection of the fluid the earthworms were released back into the container. The extract was then filtered using a syringe filter (0.22μ). The filtrate was used for the antimicrobial assay following agar dilution and disc diffusion assay¹⁰.

Agar Dilution

Muller Hinton Agar (5ml) was mixed with 2% coelomic fluid and was overlaid on 15 ml of molten agar plates. Another plate with agar alone was maintained as control. Single colony of the test organism was streaked onto the surface of the agar plate and incubated overnight at 37°C. Bacterial growth was measured on a scale of zero (no growth) to four (growth of control).

Disc Diffusion Assay

Overnight test cultures of the selected pathogens were used to develop the inoculum containing 10^8 cfu/ml and was spread over the surface of the agar plate and incubated at 37°C for 30 min. Sterile discs (6mm) were impregnated with 20 µl of coelomic fluid and placed onto the inoculated surface of the agar plates. The plates were then incubated at 37°C for 24 hours. After incubation the plates were observed and the zones of inhibition were measured. Tetracyclin (20µg/disc) and Streptomycin (20µg/disc) discs were used as the positive control.

Statistical evaluation

Antimicrobial activity test results were expressed as mean± standard deviation of the mean of the growth suppression zone diameters obtained against CF. The Student's T-test was used for comparing the results of the two techniques used. P-values lower than 0.05 ($p < 0.05$) were considered significant. It was applied to the mean activities of crude (D_0) and the 1:10 diluted CF (D_1).

RESULTS AND DISCUSSION

Well diffusion assay results (Table 1) clearly indicated that *S. aureus* was suppressed more the D_0 (Crude) CF than *B. subtilis* (6.1 ± 0.1). The zone of inhibition (18.1 ± 0.1) shown by the crude CF against *S. aureus* was almost on par with the zone produced by antibiotic tetracyclin. A further concentrated CF could show suppression similar to tetracyclin. Coelomic fluid is more effective against *S. aureus*. The spore-former *B. subtilis* showed least sensitivity to CF unlike the non-spore former *S. aureus*. The differences in the means within groups were analyzed by ANOVA. The results of the crude CF (D_0) were highly significant at $p < 0.01$. Antibacterial activities showed significant loss in the activities after the CF dilution.

Table 1: Antibacterial assay by disc diffusion indicating the zone of inhibition (mm)

Organisms	D0	D1	t-value	Tetracyclin	Streptomycin
<i>Staphylococcus aureus</i>	18.1 ± 0.1	5.06 ± 0.1	1.5631E+162	21 ± 0.5	23 ± 0.5
<i>Bacillus subtilis</i>	6.1 ± 0.1	1.85 ± 0.16	6.0119E+161	19 ± 0.5	24 ± 0.5

Mean±SD, n=6 D₀ Undiluted Coelomic fluid, D₁ CF 1:10 diluted in Holtfreter's earthworm saline solution

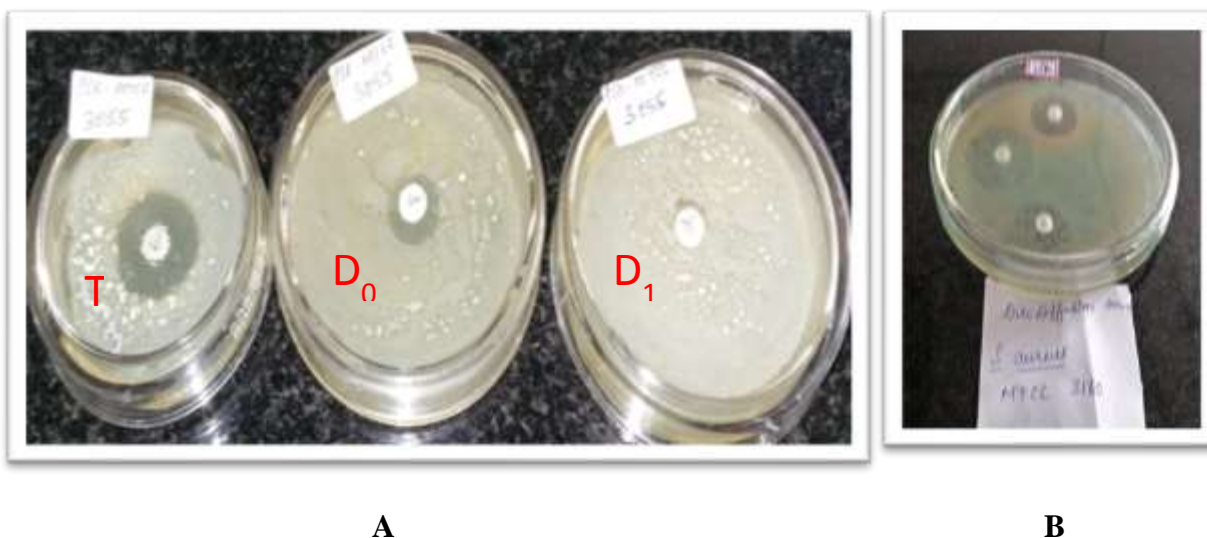


Figure 1: Zone of inhibition of A. *Bacillus subtilis* B. *Staphylococcus aureus* against coelomic fluid of the earthworm, *Eudrilus eugeniae* in disc diffusion assay

Similar bacterial suppression was reported when the study was carried out by using the powder prepared from dried and crushed entire earthworm,¹¹ and CF of the earthworm, *Lampito mauritii*¹². Earthworm paste prepared from *Eudrilus eugeniae* was also shown to be antibacterial and antifungal¹³. Similar antibacterial activities of CF of EE were reported against bacteria and fungi¹⁴. Powder prepared from dried earthworm, *Perionyx excavates* showed antibacterial activity higher than *L. mauritii*.¹⁵ The antibacterial activity in the earthworm, *Lumbricus rubellus* was attributed to an antimicrobial peptide lumbricin I¹⁶

In the agar dilution assay results (Table 2) there was 50% suppression of both the test organisms when compared to the growth in the control plate without the CF.

Table 2: Suppression of the test organisms by Agar dilution method

<i>Agar Dilution assay</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
D ₀	++	++
D ₁	+++	+++
Control	++++	++++

D₀ Undiluted Coelomic fluid, D₁ CF 1:10 diluted in Holtfreter's earthworm saline solution The growth of organisms represented using symbols (+). +++++ represents no suppression

Method followed to study the effect of CF and concentration of CF to check the suppression influences the results. In case of agar dilution the entire surface containing the CF and is exposed

to the organism whereas in disc diffusion technique it takes time for diffusion of CF into the media.

With the advancement of the biochemical techniques in the last few decades, research studies on the therapeutic effects of earthworms have increased¹⁷. The study proved that CF has antibacterial factors that if purified would be effective in treating infections caused by *S. aureus* whereas the activity was of low level against *B. subtilis*. This preliminary study is an indication that there is a possibility for identifying and using the antibacterial factor from CF of earthworm to suppress these pathogens in place of prevailing antibiotics in the market.

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