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Coelomic Fluid of Earthworm *(Eudriluseugenieae)* as Antibacterial Agent Against *Vibrio Alginolyticus* and *Staphylococcus Hominis*

KEYWORDS: Coelomic fluid, antibacterial agent, *Vibrio alginolyticus, Staphylococcus hominis*

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ABSTRACT *Coelomic fluid (CF) of earthworm Eudrilus eugeniae was collected and its antibacterial activity was tested against two strains of bacteria Vibrio alginolyticus and Staphylococcus hominis procured from IMTECH, Chandigarh. The gut of the earthworm was cleared by feeding on filter paper and CF was collected by chemical method. Dilution assay, agar dilution assay, disc diffusion assay and agar diffusion assays were carried out to test the antibacterial activity. Results indicate the suppression of growth of both the strains of bacteria. However, response of S. hominiswas better than that of Vibrio. Dilution assay proved to be the most sensitive method for antibacterial comes in direct contact with CF during the assay.*

Resistance to antibiotics by bacteria has become a big threat. Penicillin resistance rates for different strains of bacteria are reported in Europe and Asia (Baquero, 1995). Resistance to methicillin in *Staphylococcus aureus* has been reported worldwide (Voss, 1994). Thus the need to search for drugs derived from different sources has increased in recent years. Invertebrate species have developed a variety of defense mechanisms efficiently by recognizing and responding to nonself substances (Little, 2005). Earthworms have proved to be invertebrate models for immunologists in the early sixties (Cooper, 1996). *Aeromonas hydrophilaand Bacillus megaterium were* demonstrated to be sensitive to coelomic fluid of the earthworm, *Eiseniafetida Andre* (*Valembois et al.,* 1982).It also exhibits strong hemolytic activity (Du Pasquier and Du-prat 1968). Bakti *et al.*, (2003) demonstrated that the extracts of the earthworm, *Pontoscolex corethrurus has* antimicrobial activity. Aqueous extracts of earthworm *E. eugeniae are* antimicrobial to plant *pathogens; Xanthomonas campestris and Fusarium oxysporum* (Shobha and Kale, 2006).Thus effectiveness of CF of earthworms has been shown to have ability to suppress the proliferation of some of the known plant and animal pathogens. The aim of the present study was to find out the sensitivities of human pathogens to coelomic fluid of *E. eugeniae*.

Materials and Methods:
S.hominis(MTCC 4435) and *V. alginolyticus*(MTCC 4439) were procured from IMTECH, Chandigarh. They were revived and maintained on Nutrient agar medium. The earthworm Gut was cleared of the organic waste by allowing them to feed on wet ordinary filter paper for 48 hours by keeping them in a glass container covered with aluminium foil with pinholes for proper aeration.After clearing the gut CF was collected by chemical method (*Brousseau et al.,1997). About*15 – 20 ml of the fluidwas collected from 100 worms.The extract was then filtered through a 0.22µ sterile Whatmanno. 1 filter paper. The filtrate was used for the antimicrobial assay.

Antimicrobial Assays:
Influence of the coelomic fluid on the test cultures was studied following different techniques.

Dilution Assay:Test organisms were prepared in physiological (0.85%) salt solution to McFarland 0.5 standard (1 X 10^8 CFU ml^{-1}). The coelomic fluid filtrate was diluted at ratios of 1:10; 1:100 and 1:250 in Holtfreter's earthworm saline solution.Bacterial suspensions were mixed with undiluted and diluted filtrate at a ratio of 1:1 (v/v). These aliquots were incubated at 37°C for 16 hours. After incubation, bacterial counts of all the dilutions were determined by SPC on Muller Hinton agar. Suspensions in Holtfreter's earthworm saline solution were used as control. Coelomic fluid was considered efficient when bacterial inhibition wasatleast 50% (Hammer *et al.*, 1999).

Diffusion Assay: Bacterial suspension was prepared in saline to a McFarland 0.5 standard(1X10^8 CFU/ml) and spread on Muller Hinton Agar. Suspensions were loaded in the wells. The plates were incubated at 37°C for 24 hours. Zones of bacterial inhibition were recorded.

Agar Dilution assay: Nutrient agar(5ml) was mixed with 2% coelomic fluid and was overlayed 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. Bacterial growth was measured on a scale of zero (no growth) to four (growth of control) (Hood *et al.*, 2003).

Disc Diffusion Assay: Overnight test culture (0.5ml) was spread over the surface of the agar plate and incubated at 37°C for 30 min. Sterile discs (6mm) were impregnated with 10 µl of coelomic fluid and placed onto the inoculated surface of the agar plates. Plates were incubated overnight at 37 °C and the zones of inhibition were observed (Hood *et al.*, 2003).

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Statistical Analysis: The data collected in dilution assay was subjected to statistical analysis. Students't-test was applied to determine the significance of dilution on the growth of bacteria.

Results and Discussion:

Analysis of the results (table 1) indicates that *S. hominis* is more suppressive to the CF of the earthworm, E. eugeniaewhen compared to *V. alginolyticus*. In antibacterial activity assays with dilution technique, it was observed that all concentrations of suspensions of CF of E. eugeniae significantly inhibited the growth of bacteria. The crude extract was most effective against *S. hominis*. In the diffusion assay the highest diameter of inhibitory zone (14±0.5mm) was observed against S. hominis followed by *V. alginolyticus*(13.2±0.2 mm) at 10^8 cfu mL^{-1}. The dilution assay method of determining the antibacterial activity was noted to be the most sensitive of all the different methods done. The activity might be attributed to the bacteria coming directly in contact with the CF. In diffusion assays, the concentration of CF might be affected as it passes through the medium.

Suppression of bacteria by the CF ofthe earthworm, *E.foetida* was reported *inin vivo* studies (Lange, 1997; Weng, 2004). Similar antibacterial activity of coelomic fluid of earthworms from different localities at Istanbul are reported based on dilution and agar diffusion assays against Gram negative and Gram positive bacteria (Arslan-Aydogdu *et al.*,2008). Rivai Bakti *et al.*, (2003) studied the antimicrobial activity of earthworm extract (*Pontoscolex corethrurus*) in different solvents and reported the antibacterial activity of the extracts on S. aureus and Escherichia coliin terms of the inhibition zones. Ansari *et al.*,, 2011; Anita *et al*, 2013 in their studies have used earthworm powder to test the antibacterial activity. However, in the present study it is only the CF at minimal concentrations that has shown suppression. This clearly indicates that the CF is the best source to look for the biomolecule that can be used for suppressing the activity of pathogens.

Table 1Suppression of growth of *Staphylococcus hominis* and *Vibrio alginolyticusin* presence of different concentrations of coelomic fluid of earthworm *Eudrilus eugeniae* (n±SE; where n=6)

Assay	Bacteria	Undiluted	1:10	1:100	1:250	Control	Tetracyclin
Dilution Assay (x X 10^4) cfu	*Staphylococcus hominis*	51±1.52	83.6±1.2	*102±1.46	NS128.3±1.5	199±1.64	TLTC
	Vibrio alginolyticus	83.66±.57	*101.33±1.54	151.6±1.52	194.33±1.08	224±1.52	TLTC
Diffusion Assay (mm)	*Staphylococcus hominis*	14±0.5	2.86±0.05	1.67±0.15	0.43±0.11	0	18
	Vibrio alginolyticus	13.2±0.2	1.83±0.05	1.16±0.05	0.43±0.05	0	15
Agar Dilution Assay	*Staphylococcus hominis*	2±0	2.5±0	3±0	3±0	4±0	0
	Vibrio alginolyticus	3±0	3±0	3.5±0	4±0	4±0	0
Disc Diffusion Assay (mm)	*Staphylococcus hominis*	13.06±0.11	2.53±0.05	1.23±0.05	0.76±0.15	0	20
	Vibrio alginolyticus	12.56±0.05	1.93±0.05	0.86±0.05	0.4±.05	0	16

***Significant NS Not significant TLTC-Too Low To Count**

Conclusion:

The results indicate the possibility of bringing about the suppression of the *Staphylococcus* and *Vibrio spp.* It can pave way for purifying the biomolecules to introduce into the field of pharmaceuticals for external use on domestic animals and human beings

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