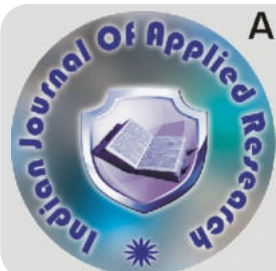


Research Paper

Biotechnology



Amino acid profile, Anti coagulant and Anti oxidant activities of coelomic fluid of the Earthworms *Polypheretima elongata* and *Eudrilus eugeniae* in relation to medical application

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ABSTRACT

*Production of pharmacologically important compounds from earthworms is a novel area in modern day medicine termed as vermiceuticals. In the present study effect of coelomic fluids of *Polypheretima elongata* and *Eudrilus eugeniae* were independently examined for one of their possible medical applications. Coelomic fluids from *P.elongata* and *E.eugeniae* were extracted by applying heat and cold shock treatments. The coelomic fluids of both the earthworm species showed anti oxidant and anti coagulant activities at different levels. Anti Coagulation assay was performed to the coelomic fluids of the earthworm species using standard clotting time delay method of Baughmann (1995). Both the species *P.elongata* and *E.eugeniae* exhibited the anticoagulant activity with the clotting time delay of maximum 60 and 40 Seconds respectively with the coelomic fluids volume of 200 μ l. Both the species exhibited antioxidant activity. The presence of Glutathione was confirmed in both the species at a concentration of 0.12 μ g/ml and 0.06 μ g/ml respectively in *P.elongata* and *E.eugeniae*. The above results clearly indicate that coelomic fluid of *P.elongata* has bioactive molecules in higher levels than *E.eugeniae*. The salient findings of the study include coelomic fluid of earthworms namely *P.elongata* and *E.eugeniae* has protein factors and some of the essential amino acids which have anticoagulant and antioxidant activity. Further research on the principal active components of coelomic fluids of two above mentioned earthworm species leads to development of drug against enhancement of wound healing property, to treat cancer, thrombolytic and hemophilic disorders in humans.*

Key word : *Polypheretima Elongata*, *Eudrilus Eugeniae*, Coelomic Fluid

Introduction

The body fluid filled in the space between body wall and gut is called coelomic fluid. The coelomic fluid of earthworms contain various molecules which exhibit important biological properties such as antimicrobial substances¹⁻⁴, hemagglutinating⁵ and anticoagulant agents⁷. The primary function of the coelomic fluid presumably is to destroy membranes of foreign cells, a mechanism that causes cell death by cytosol release and is attributed to coelomocytes which secrete humoral effectors into the coelomic fluid. The components of the coelomic fluid are lectin in character⁷⁻⁹.

The coelomic fluid is known to have antibacterial activity and it was shown that it inhibited the proliferation of *Xanthomonas campestris*, *Ralstonia solanacearum* and *Fusarium oxysporum*. It was also confirmed that antimicrobial activity existed in coelomic fluid was essentially due to fetidins, a protein. Earthworm immune system is known to have proteins and peptides and these cause cell lysis.

The present study reveals the comparative characterization of coelomic fluids of *Polypheretima elongata* and *Eudrilus eugeniae* with respect to the factors responsible for anticoagulant and anti oxidant activity.

Materials And Methods

Polypheretima elongata are endogeic earthworms and are collected from the banana field at Srinivasapur, Karnataka, India and *Eudrilus eugeniae* the epigeic earthworms were obtained from Jayanth vermi compost, a commercial vermiculture unit in Maddur, Karnataka, India.

The chemicals used for analyses were of analytical grade.

Culturing of Earthworms

Polypheretima elongata were fed with soil and organic substances on suitable bedding in plastic trays. *Eudrilus eugeniae* were cultured on partially decomposed organic matter in plastic trays. The feed stock was cow manure decomposed for three weeks¹⁰. Moisture content of 75%-80% was maintained in culture trays.

Extraction of Coelomic fluid

Extraction of coelomic fluid from chosen earthworm species was collected according to the method of 12. The earthworms were washed with cold water 3-4 times at room temperature and their body surface was dried on filter paper. Heat shock was given by using 35°C 40°C water in suitable container and cold shock by using ice cubes in a beaker, which was rubbed against the body of the earthworms placed in dry Petri plates. The procedure is repeated for 3- 4 times. The coelomic fluid obtained from the body of the earthworms was then collected and stored in vials.

Purification of protein by Ammonium sulphate precipitation and Dialysis:

Measured volumes of coelomic fluids of both the species were taken, ammonium sulfate was added and stirred to give 35%, 45%, 55% saturation for 10 minutes respectively, later it was subjected to centrifugation at 3000 RPM for about 10 minutes.

Precipitate obtained from 35%, 45% and 55% Ammonium sulphate saturations were dissolved separately in phosphate buffer and subjected to dialysis. The samples were placed in the dialysis bags separately and the bags were placed in the beaker containing distilled water for overnight to remove excess salts, later the bags were placed in 1% sucrose solution to remove excess water for 4-5 hours. The samples were removed carefully from the dialysis bags and stored at 4°C until use. Later protein was measured by the method of Lowry et al., (1951)13 using bovine serum albumin standard (0-75µg).

Biochemical tests were carried out for screening of various amino acids in coelomic fluids of the earthworms *Polypheretima elongata* and *Eudrilus eugeniae*.

Estimation of Anticoagulant activity

Assay for anticoagulation is based on clotting time delay method of Baughmann (1968). For concentration dependent assay, different concentrations (50, 75, 100, 150, 200 µl) of protein samples were added to 0.1 ml thrombin (10 NIH U/ml) solution. The samples were incubated for four minutes at 37°C. Later 0.5ml of 0.5% fibrinogen was added. The time taken to form a clot in each concentration was recorded.

Estimation of Antioxidant activity by standard Glutathione method

Glutathione is one of the antioxidant. Different concentrations of coelomic fluids were added to 3ml of precipitating solution, mixed well and allowed to stand for 5 min. To two ml of filtrate 8ml of phosphate solution, 1ml DTNB were added and mixed. Glutathione reduces 5-5' dithiobis (2- nitro benzoic acid) to yellow colored TNB. Color intensity is measured at 412 nm.

Results And Discussion

Polypheretima elongata and *Eudrilus eugeniae* coelomic fluids exhibited the highest protein concentration 158 and 90 µg/ml respectively at a saturation of 45%, as shown in the (Table 1).

Qualitative tests for screening of essential amino acids in coelomic fluids of the earthworms *Polypheretima elongata* and *Eudrilus eugeniae* exhibited the presence of amino acids proline, serine, tyrosine and tryptophan. Indole group and phenolic amino acids are absent in coelomic fluid of *Eudrilus eugeniae*. Sulphur containing amino acids are absent in both the species (Table 2). The results revealed the presence of molecules that exhibit the anticoagulant activity in the coelomic fluid of *Polypheretima elongata* and *Eudrilus eugeniae* (Table 3). The anticoagulant activity is concentration dependent (Fig 1). This result is in par with the result obtained by earlier workers (Jeon and Woon-Joon, 1995). The coelomic fluid of both the species exhibited the anti oxidant activity (Table 3). The anti oxidant activity is concentration dependent shown in the (Fig 2).

Table 1: Protein levels in the purified coelomic fluids of *P.elongata* and *E.eugeniae*

Sr No	Ammonium Sulphate Saturation	Protein µg/ml	
		<i>P.elongata</i>	<i>E.eugeniae</i>
1	45%	158	90
2	55%	40	30
3	60%	36	21

Table 2: Qualitative tests for screening of essential amino acids in coelomic fluid of the earthworms *Polypheretima elongata* and *Eudrilus eugeniae*

Test	Observation	<i>P.elongata</i>	<i>E.eugeniae</i>
Ninhydrin test:	Appearance of purple to blue color	Positive, presence of serine (α-amino acids)	Positive Presence of serine
Xanthoproteic test:	Orange color	Positive, Presence of aromatic amino acids	Positive, Presence of aromatic amino acids
Pauly's Diazo test:	Appearance of red color	Positive, Presence of tyrosine/Histidine	Positive, Presence of tyrosine/Histidine
Millons test:	Appearance of brick red color.	Positive, Presence of phenolic amino acids	Negative, Absence of phenolic amino acids
Hopkins-cole test:	Appearance of a purple color at the interface	Positive, Presence of amino acids with indole group.	Negative, Absence of amino acids with indole group
Lead sulphite test:	Appearance of a black precipitate	Negative, Absence of sulphur containing amino acids	Negative, Absence of sulphur containing amino acids

Table 3: Anti Coagulant Activity

Sr. No	Coelomic fluid (µl)	Time delayed to clot (Seconds)	
		<i>P.elongata</i>	<i>E.eugeniae</i>
1	50	5 ± 0.7	3.5 ± 0.35
2	75	10 ± 0.5	8 ± 0.7
3	100	20 ± 0.7	15 ± 1.4
4	150	35 ± 1.4	26 ± 1.5
5	200	60 ± 2.12	42 ± 1.4

Table 4: Amount of glutathione present in coelomic fluid of the earthworms *Polypheretima elongata* and *Eudrilus eugeniae*

	<i>Polypheretima Elongata</i>		
	0.5ml	1ml	2 ml
Volume of the sample			
Amount of glutathione (µg/ml)	0.08±0.007	0.12±0.014	0.23±0.02
	<i>Eudrilus eugeniae</i>		
	0.5	1	2
Volume of the sample			
Amount of glutathione (µg/ml)	0.03±0.007	0.06±0.014	0.13±0.02

Figure 1: Clotting time delay with increase in concentration of coelomic fluid of *P.elongata* and *E.eugeniae*.

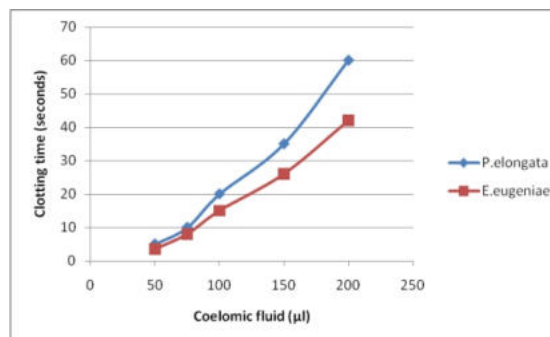
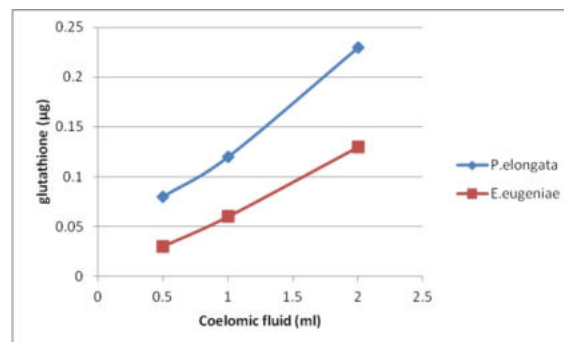


Figure 2: Amount of glutathione present in coelomic fluid of *P.elongata* and *E.eugeniae*



Coelomic fluid of *Polypheretima elongata* exhibited higher antioxidant activity when compared to the coelomic fluid of *Eudrilus eugeniae*. The anticoagulant and antioxidant hemolytic activities may be due to peptides called as cytolytins present in the coelomic fluid of two species of earthworms¹⁴.

The outcome of the study supports for recommending coelomic fluid as a feed additive for poultry and aqua culture. The antioxidant property can be used to increase the cell

longevity prevention of ageing in humans and cell culture systems. The anticoagulant property can be used to develop a commercial formulation used to prevent the blood clots during surgery in future after some more investigations on the actual principles responsible and with further purification

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