



EVALUATION OF ANTIFUNGAL ACTIVITY IN DIFFERENT EPIGEIC EARTHWORM SPECIES

Zoology

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ABSTRACT

Medicinal value of earthworms has been known since centuries, this is evident from history of ancient southeastern countries like China, Japan and Vietnam. The present study was carried out to evaluate antifungal activity by using different epigeic earthworm species, *Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus* and standard drug Itraconazole as positive control against *Candida albicans*. The agar well diffusion method was used for antifungal assay maintained at 37 °C for 24 hrs. The results of the present study revealed that antifungal activity i.e. Zone of Inhibition was more in *Eudrilus eugeniae* (EE) followed by *Perionyx excavatus* (PE) but no antifungal activity was observed in *Eisenia fetida* (EF). Again zone of inhibition in EE was comparatively more (15.33 ± 0.33) at $10 \mu\text{g/ml}$ concentration than that of $20 \mu\text{g/ml}$ (10.33 ± 0.33) and $30 \mu\text{g/ml}$ (10.66 ± 0.33) respectively, whereas antifungal activity was only seen in $30 \mu\text{g/ml}$ (11.33 ± 0.33) in PE. Further, antifungal activities of EE & PE were comparatively less than that of standard drug (Itraconazole) as positive control at $5 \mu\text{g/ml}$ in the present study. Based on the results, it can be concluded that the samples of EE showed much higher antifungal activity as compared to with other two species PE & EF. The present study also suggests that the biomolecules or bioactive compounds present in particular earthworm tissue are going to influence against various pathogenic microorganisms, such earthworms can be useful in further purification of biomolecules that can be used for new drug discovery in pharmaceutical applications.

KEYWORDS

Antifungal activity, Epigeic earthworms, *Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus* and *Candida albicans*.

INTRODUCTION:

Earthworms plays a major role in the proper functioning of the soil ecosystem as they act as scavenger and helps in recycling of nutrients by using dead and decaying organic materials. Earthworms are believed to act as regulators of various microorganisms in the soil ecosystem as these creatures and other soil invertebrates are relatively long living organisms and rich in microorganisms, fungi and other potential pathogens.

Earthworms have been used successfully as medicine for various remedies since 1340 AD.^[1, 2] this is evident from the ancient southeastern asian medicine (China, Japan, Vietnam). The earthworms are known to have important source of biomolecules such as protein, amino acids, peptides, enzymes and other physiologically active substances that have been used in traditional practices for the treatment of numerous diseases.^[3] The antitumour activities of earthworm fibrinolytic enzyme (EFE) on human hepatoma cells were studied by Hong.^[4] The anti-inflammatory and anti-pyretic activities of biologically active extract/compound isolated from earthworm, *Lampito mauritii* were also determined by Balamurugan et al.^[5]

Earthworms have been used for reducing fever due to its antipyretic activity and also used for testing pregnancy in human females.^[6] They were also found useful in curing chronic cough, jaundice, diarrhea and for facilitating delivery^[7]. Hori et al.^[8] have reported significant antipyretic activity of bioactive molecules derived/ extracted from earthworms. The bioactive compounds/ molecules from earthworms exhibited different biological activities such as fibrinolytic, anti-coagulative, anti-cancer, anti-microbial, activities thus, may be exploited for the treatment of variety of diseases.^[9]

Further, the potential medicinal value of earthworms (*Lumbricus rubellus* and *Eisenia fetida*) for the treatment of heart diseases have brought revolution in the vermitechology.^[10,11] Researchers are currently looking into earthworms for anti-inflammatory properties for treating arthritis and other joint ailments. Recently, earthworm tissues and their coelomic fluid are known to have cytolytic, agglutinins, proteolytic, hemolytic, anti-pyretic, tumor static and anti-microbial properties etc.

Much work on these aspects is being carried out with respect to only few species of earthworms such as *Lumbricus terrestris*, *Lumbricus rubellus* and *Eisenia fetida*, *Lampito mauritii*. Now, the researcher are concentrating in identification and isolation of various bioactive compounds/ molecules from different earthworm species as there are more than 6000 species of earthworms living in different habitats of

environment. Hence, the present study aims to evaluate the antifungal activity of different epigeic earthworm species such as *Eudrilus Eugenia* (EE), *Eisenia fetida* (EF) and *Perionyx Excavatus* (PE) against the fungal strain, *Candida albicans* by using well diffusion method.

MATERIALS AND METHODS:

I. Collection and culturing of earthworms:

Adult epigeic earthworms such as *Eudrilus Eugenia* (EE), *Eisenia fetida* (EF) and *Perionyx excavatus* (PE) were obtained from UAS, Dharwad were stock cultured and maintained at Department of Studies in Zoology, Karnatak University, Dharwad (Karnataka), India.

II. Preparation of earthworm powder:

The earthworm powder was prepared as per the method prescribed by Shradha and Pratik^[12] with slight modifications. About 20-30 sexually matured all three epigeic earthworm species were washed separately in running tap water to remove any dirt from the body surface, then these earthworms were fed with soaked tissue paper for about 24 hours so as to clean their gut properly. The gut cleared earthworms were again washed in distilled water, then kept them in tightly covered petriplates and were exposed to sunlight for drying. The whole dried earthworms were crushed using mortar and pestle so as to produce brown colored powder and then it is stored in an air tight plastic cover and then used it for preparation of samples for assessment of antifungal activity.

III. The fungal strains used:

Pure cultures of *Candida albicans* are used to assess antifungal activity of various concentration of different epigeic earthworm species (EE, EF, PE) along with standard drug, Itraconazole as positive Control.

IV. Media preparation:

The necessary optimized media using Dextrose+ Peptone+ KNO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$ + CaCl_2 and Agar was prepared by dissolving them in distilled water and was autoclaved at 15lbs pressure at 121°C for about 15 minutes. The autoclaved media was mixed well and 25ml of it was poured/ onto 100mm petriplates so as to culture fungal strain, *C. albicans* (NCIM-3012).

V. Antifungal activity:

Petriplates containing 25 ml of optimized media were inoculated with 24 hrs old cultures of *Candida albicans* strain (NCIM-3102) using bent glass rod separately. Spread plate method was followed and well was made using well borer. Test concentrations of 10, 20, $30 \mu\text{g/ml}$ was loaded with each plate along with fungal strain $40 \mu\text{l/ml}$ well respectively. Then plates were incubated for 24 hrs at 37°C (Table -1). The antifungal activity was observed and confirmed by measuring the

diameter of zone of inhibition(mm) formed around each well.

Table1. Experimental design used for studying antifungal activity through zone of inhibition (mm) against the fungal strain, *Candida albicans* (NCIM-3102).

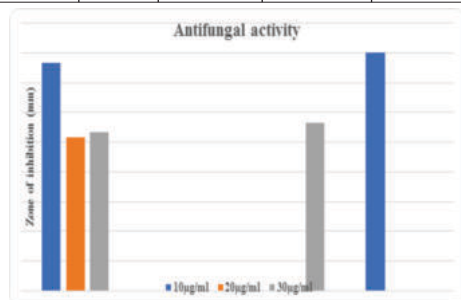
Sl. No.	Test conc. (µg/ml) of earthworm samples	Optimized media (Dextrose+ Peptone+ KNO ₃ , NH ₄ H ₂ PO ₄ + CaCl ₂ and Agar)	Fungal culture (24hrs old) loaded in /well	Incubation time & temperature
1	10µg/ml	25ml	40 µl/ml	For 24hrs at 37°C
2	20µg/ml	25ml	40 µl/ml	
3	30µg/ml	25ml	40 µl/ml	

RESULTS:

The data of the present study was represented in Table-2, Graph-1 and Figure-1-3. The antifungal activity of various test conc. of earthworm samples & std drug were determined by measuring the diameter of the zone of inhibition (mm) and were photographed. The results revealed that the antifungal activity of EE was much prominent followed by PE and it was nil in case of EF against the fungal strain, *Candida albicans*. Further, zone of inhibition (mm) in EE for 10, 20 and 30 µg/ml test concentrations were 15.33±0.33, 10.33±0.33 and 10.66±0.33 respectively, whereas in PE it was only at 30µg/ml with 11.33±0.33 zone of inhibition and nil at 10 & 20µg/ml test conc. There is as such no antifungal activity was seen in EF as it was evident by no zone of inhibition with any test concentrations (10, 20 & 30µg/ml). The std. drug Itraconazole (5µg/ml) was used as positive control and possessed maximum antifungal activity with zone of inhibition(mm) of 16.00±0.35 (Table-2; & Graph-1 & Figure 1-3) in comparison with different test conc of earthworm.

Table 2. Antifungal activity with various test conc of different epigeic earthworm species (EE, EF and PE) along with standard drug, Itraconazole as positive control against the fungal strain, *Candida albicans* measured by zone of inhibition (mm) formed around the well. Data are expressed in Mean±SE.

Sl No.	Epigeic Earthworm species	Zone of Inhibition (mm)			Std. Drug (Itraconazole) as Control (5µg/ml)
		10µg/ml	20µg/ml	30µg/ml	
1	<i>Eudrilus eugeniae</i>	15.33±0.33	10.33±0.33	10.66±0.33	16.00±0.35
2	<i>Eisenia fetida</i>	-	-	-	
3	<i>Perionyx excavatus</i>	-	-	11.33±0.33	



Graph 1. Antifungal activity of various test concentration of different epigeic earthworm species along with standard drug as positive control.



Figure 1. Determination of antifungal activity through zone &

inhibition(mm) with different sample 1,2, 3 of *Eudrilus eugeniae* represents 10, 20, and 30 µg/ml respectively and standard drug Itraconazole (5 µg/ml) as positive control.



Figure 2. Determination of antifungal activity through zone of inhibition with different samples 1,2,3 of *Eisenia fetida* represents 10, 20, and 30 µg/ml respectively and standard drug Itraconazole (5 µg/ml) as positive control.



Figure 3. Determination of antifungal activity through zone of inhibition with different samples 1,2,3 of *Perionyx excavatus* represents 10, 20, and 30µg/ml respectively and standard drug Itraconazole (5 µg/ml) as positive control.

DISCUSSION:

The present study clearly indicates that the epigeic earthworm, EE contains good percentage of antifungal potency, followed by PE. This is attributed to the presence of various bioactive compounds in the earthworm tissues of EE & PE against pathogenic fungal strains.

The variations in antifungal activities of different epigeic earthworm species as witnessed in the present study may be due to difference in the presence of active biological molecules in the tissues or coelomic fluid of these earthworm species and type of environment in which they live and it may also depends on the particular fungal strains used in the study.

Khomyakov et al^[13] have found that antimicrobial agents of digestive fluid of worms are found in the earthworm body but not in the soil microorganisms entering their digestive gut. Shobha and Kale^[1] have reported about the gut extracts of earthworms have antibacterial and antifungal activity. Even Engelmann et al^[14] and Balamurugan et al^[5] have also found that earthworm coelomic fluid contains biologically active molecules and leukocytes that participate in phagocytosis, encapsulation and killing of HeLa, HEP-2, PA-12 and PA 317 cells *in-vitro*. Prakash and Gunasekaran^[15] have reported that earthworm powder was used against various ailments in indigenous system of medicine, which was found to be a good remedy against various pathogenic microorganisms.

The coelomocytes of earthworms intensively synthesize and secrete proteins that adhere to the bacteria and other microorganisms, forming aggregate and may inhibit their further proliferation.^[16] *Eudrilus eugeniae* and *Perionyx excavatus* are the two earthworm species in our studies are having a source to look for important biomolecules that can be used precisely to suppress the activity of different pathogenic microorganisms.^[17] The antibacterial and antifungal activities of the whole tissue extract of earthworm, *Eudrilus eugeniae* was also tested by (Chauhan et al^[18]) using well diffusion method

The results of the present study revealed that antifungal activity (Zone of Inhibition) was more in EE followed by PE and no antifungal activity was seen in EF. Further, 10µg/ml test concentration of EE was comparatively more effective (15.33±0.33) than that of 20 and 30

µg/ml as it was 10.33 ± 0.33 and 10.66 ± 0.33 respectively and the same was observed at $30 \mu\text{g/ml}$ only in case of PE (11.33 ± 0.33). Further, all the above activities were comparatively less than that of standard drug, Itraconazole as positive control at $5 \mu\text{g/ml}$ in the present study.

CONCLUSION:

Based on the result of the present study, it can be concluded that the earthworm, *E.euegniae* showed promising antifungal activity followed by *P. excavatus* at different test concentrations against *C. albicans*. This study suggests that the tissue extracts of earthworm species EE and PE might be having useful bioactive molecules for new drug design in pharmaceutical applications. Further, it can also have way for purification and extraction of important bioactive compounds present in these earthworms' tissues is necessary in future.

REFERENCES:

- Shobha SV, Kale R. Antimicrobial potency of earthworm, *Eudrilus eugeniae* on certain plant pathogens. *Journal of Agricultural Technology*.2007;8(2):403-415.
- Hossam, El-Din Mohamed Omar, Zedan Z, Ibraheim, Nasser A, El-Shimy, Rouwaida SA. Anti-inflammatory, antipyretic and antioxidant activities of the earthworms extract. *J. Biol. Earth Sci*. 2012;2:1.
- Wenli L, Chong W, Zhenjun S. Vermi-pharmaceuticals and active proteins isolated from earthworms. *Pedobiologia*. 2011;54S:S49-S56.
- Hong C. Earthworm fibrinolytic enzyme and antitumoractivity on human hepatoma cells, invitro and in vivo. *Chinese Med. J*.2007; 260-264.
- Balamurugan M, Parthasarathi K, Cooper EL, Ranganathan LS. Anti-inflammatory and anti-pyretic activities of biologically active extract isolated from whole earthworm, *Lampito mauritii*. *J.Ethnopharmacol*.2008;121(2):330-332.
- Hassenbein GA. Pregnancy test on earthworms. *Arch. Gynakol*. 1951;181:5-28
- Wahid A, Siddiqui HH. 1961. A survey of drugs -2111 edition. *Institute of History of Medicine and Medical Research, New Delhi*. 1961:16-17.
- Hori M, Kondon K, Yoshida T, Koshih E, Minami S. Studies of anti-pyretic components in Japanese earthworm. *Biochem. Pharmacol*. 1974; 23:1582-90.
- Cooper EL, Balamurugan M, Huang CY, Tsao CR, Heredia J, Tommaso- Ponzetta M. Earthworms dilong: Ancient, inexpensive, non controversial models may help clarify approaches to integrated medicine emphasizing neuroimmune systems. *Evid Based Complement Alternative Medicine*. 102:164-152.
- Hwang CM, Kim D, Huh SH. In-vivo evaluation of lumbrokinase extracted from earthworm, *Lumbricus rubellus* in a prosthetic vascular graft. *Cardiovascular Surgery*.2002;43:891-894
- Qingsui C. A new medicine for heart diseases containing enzyme activator extracted from earthworms. In: The Utilization of Earthworms for Health Remedies Lopez and Alis (Eds.)2003.
- Shraddha C, Pratik M. Biochemical estimation of paste prepared from the earthworm, *Pheretima posthuma* in India. *IJSRD*.2017; 5.
- Khomyakov NV, Kharin SA, Nechitailo T, Yu, Golyshin PN, Kurakov AV, Byzov BA, Zvyagintsev DG. Reaction of microorganisms to the digestive fluid of earthworms. *Microbiology*.2007;76:45-54.
- Engelmann P, Kiss J, Cooper EL, Nemeth P. Earthworm leukocytes kill HeLa, HEp-2, PC-12 and PA317 cells in-vitro. *J. Biochem. Biophys. Methods*.2014; 61:215-228.
- Prakash M, Gunasekaran G. Antibacterial activity of the indigenous earthworms *Lampito mauritii* (Kinberg) and *Perionyx excavatus* (Perrier). *The J. Alterna. and Complemen. Med*. 2011;17(2): 167-170.
- Marta J, Fiolka Mirosław P, Zagaja Tomasz D, Piersiak Marek W, Róbel, Jarosław P. Gut bacterium of *Dendrobaena veneta* (Annelida: Oligochaeta) possesses antimycobacterial activity. *J. Invertebr. Pathol*.2010;105:63-67
- Kulakarni,P, Kaicker G. Coelomic fluid of earthworm, *Eudrilus eugeniae* as antibacterial agent against *Vibrio alginolyticus* and *Staphylococcus hominis*. *Ind. J . Appl Res*.2014;4(12):462-463.
- Chauhan SP, Tomar J, Prasad BKS, Agarwal P. Evaluation of antimicrobial activity of earthworm, *Eudrilus eugeniae* tissue extract. *Journal of Chemical and Pharmaceutical Research*. 2014; 6(8): 28-38.