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Original Research Article

Antimicrobial activity of Earthworm Powder (*Lampito mauritii)*

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A B S T R A C T

Keywords

Lampito mauritii; antimicrobial activity; disc diffusion; petroleum ether

In the present study, various solvent extracts of dried earthworm powder (*Lampito mauritii)* were prepared and subjected to preliminary screening for antimicrobial activity, which was determined by disc diffusion method. It was found that 95% ethanol extract of earthworm was potent antibacterial agent against *Aeromonas hydrophila* and antifungal agent against *Candida albicans*. The petroleum ether and aqueous extract of earthworm powder possessed minimum antifungal activity in comparison with ethanolic extract. These studies may lead to the formulation of new antimicrobial drug.

Introduction

Earthworm plays a major role in the proper functioning of the soil ecosystem. It acts as scavenger and helps in recycling of dead and decayed plant material by feeding on them. Earthworm increases the soil fertility and is often referred as farmer's friend. Earthworms have been used in medicine for various remedies since 1340 AD and recognized in oriental medicine as anti- inflammatory, analgesic and antipyretic agent. It shows anticancer effect by preventing excess glucose uptake. In ancient Burma and Laos, smallpox victims were bathed with water where earthworms had been soaked. Similarly worms were boiled in water with salt and onions and the broth given to women with postpartum weakness or difficulty nursing. In Iran dried earthworms were prescribed to treat jaundice.

Earthworms are relatively long living organisms in an environment rich with micro-organisms, fungi and other potential pathogens. As substrate feeders they have a relatively high take up of these organisms. Microorganisms are known to play a major role in soil characteristics and invertebrates are believed to act as regulators of antimicrobial activity. Earthworm surface excreta were found to have potent antimicrobial activity. Their successful survival under these conditions is supported by efficient innate immune mechanisms based on cellular activities of coelomocytes and humoral immune proteins, both components of the

earthworm coelomic fluid (CF). Earthworms respond to microbial infection through cellular and humoral defense mechanisms such as antimicrobial protein secretions. Most of the humoral defense proteins are synthesized in the skin itself. Cooper *et al.*, 2004 discovered that earthworms have been prominent with respect to lysis of bacteria and with other implications to disease. Anti-inflammatory activity and antimicrobial potency (Shobha and Kale, 2007) of *Eudrilus eugeniae* of earthworm extracts on certain plant pathogens were studied.

Earthworm powder can be given orally,since it has a potential application as a thrombolytic and also exerts an inhibitory effect on platelet aggregation, an anticoagulation effect and a relaxation effect for the vascular system, which are all effective for thrombotic therapy (Kim et al., 1998).Therefore, it can be concluded that earthworm powder represents a very promising agent for the treatment of thrombosis (Mihara et al., 1996). Its tonic properties make it beneficial support for the liver and other organ systems.

The bacteriolytic substances include fetidins (which have serine proteases and promote clots), lysenins, lumbricin, eiseniapore, coelomic cytolytic factor (CCF-1) and erythrocytolytic proteins. Some of these agents have been tested for antineoplastic potential. One substance like eisenin destroys human neoplastic cells from several human cancer cell lines (Cooper *et al*., 2004). In China, Korea, Vietnam and most of SouthEast Asia, Lumbricus has been used for their therapeutic benefits for thousands of years and referred as Earth Dragons. Antitumor activities of earthworm fibrinolytic enzyme on human hepatoma cells were studied (Hong, 2007). The anti-inflammatory and antipyretic activities of biologically active extract isolated from whole earthworm, (*Lampito mauritii*) were determined (Balamurugan *et al.,* 2008).

Present study focuse on anti-bacterial properties along with the anti-fungal properties in the powder of *Lampito mauritii* which may have applications indirectly in treatment of diseases related to different microbes and fungi.

Materials and Methods

Preparation of the Sample

Approximately 500 cultured earthworm, (*Lampito mauritii*) were collected and washed in running water to remove dirt from the body surface. The earthworms were soaked in distilled water for 6 to 8 hours to allow the soil in its tract to be excreted. Later the earthworms are washed thoroughly with distilled water and collected in a petri dish, which was then kept in an incubator for 24 hours at 55°C. After 24 hours earthworms are removed and pounded to make it into powder. This powder was stored in a refrigerator at normal temperature (Yegnanarayan *et al.,* 1987).

Anti microbial Study

Preparation of Crude Aqueous Extract

Accurately weighed 100 gm of powder was taken in a stainless steel vessel and mixed with 100 ml (1:1) of distilled water and boiled. After that, the mixture was filtered through standard Whatman filter paper (size no.1).Then the filtrate was evaporated on a hot plate until it reaches the concentrated quantity.

Preparation of Ethanolic and Petroleum Ether Extracts

100 gm of powder was taken and mixed with 95% Ethanol and Petroleum Ether separately. The mixture was prepared separately in different solvents and then filtered and the filtrates obtained were condensed in water-bath at 35°C and this process was repeated till sufficient quantity occurs.

Microorganism used

Pure cultures of three bacterial strains *Staphylococcus aureus, Salmonella typhi, Aeromonas hydrophila* and two *fungal* strains *Aspergillus niger and Candida albicans* were used.

Culture Media and Inoculum

The media used for microbial culture was Mueller Hinton agar (MHA).The bacterial cultures inoculated in MHA were incubated at 37°C for 18 hours. The suspension were checked to provide approximately 10^5 cfu/ml. Fungal cultures were inoculated and incubated at 37°C for 48 hours .

Determination of Antibacterial Activity:

The antibacterial activity of the extracts was determined by the disc diffusion method. (Rios *et al.*,1988). Overnight bacterial cultures were diluted in the Mueller-Hinton broth to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Four filter paper discs (Whatman No.1, 6 mm diameter) were placed on the inoculated agar surface. 200µl of the extracts were loaded on to the filter paper discs and were allowed to dry completely. Standard Antibiotic Erythromycin was placed as control. Plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicate.

Determination of Antifungal activity

The above same procedure was adopted to determine antifungal activity where the fungal cultures were kept for 48 hours to determine the diameter of zone of inhibition. Standard antibiotic Erythromycin was placed as control.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using micro dilution broth method. The earthworm powder extract prepared (100 mg/ml) were diluted to four different concentrations (50, 100, 150 and 200 µl/ml).To find out the MIC, three strains of bacteria were used *(Staphylococcus aureus, Salmonella typhi,* and *Aeromonas hydrophila*). The bacterial strains were prepared in broth.

The broth culture suspension of each bacterium (0.5 ml) was added to the test tubes containing different concentrations of earthworm paste. To the control test tube, the earthworm powder was not added, only sterile distilled water was used. The inoculated test tubes were incubated at 37°C under aerobic conditions. After 24 hours, the turbidity was evaluated. The MIC is the lowest concentration of EWP that inhibits the growth of the organism completely for all the tests including control and triplicates were maintained.

Results and Discussion

Earthworm powder prepared from *Lampito mauritii was* tested for antibacterial and antifungal activities. Three strains of bacteria viz. *Staphylococcus aureus, Salmonella typhi, Aeromonas hydrophila* were used for antibacterial assay that was determined by measuring the diameter of zone of inhibition recorded. Ethanolic extract of earthworm powder possessed maximum antibacterial activity in comparison with petroleum ether and aqueous extract against *Aeromonas hydrophila*. The observed diameter of zone of inhibition observed was 16 mm. In comparison to *Aeromonas hydrophila*, less diameter of zone of inhibition was observed against *Staphylococcus aureus* (15 mm) followed by *Salmonella typhi* (10 mm). Erythromycin was used as the positive control and it possess maximum diameter of zone of inhibition against *S. aureus and A. hydrophila* (18mm) followed by *Salmonella typhi* (12mm) (Table. 1). For antifungal assay two fungal strains *Aspergillus niger and Candida albicans* were used . Ethanolic extract of earthworm powder was found to have strong antifungal activity against *Candia albicans* in comparison to *Aspergillus niger*. The diameter of zone of inhibition observed was 12 mm followed by *Aspergillus niger* (9 mm) while petroleum ether extract of earthworm powder was ineffective against these fungal strains. Erythromycin was used as the positive control and possess maximum antifungal activity in comparison to earthworm extract (Table. 2).The present study clearly indicate that earthworm powder contains good antimicrobial potency. The MIC results indicated that earthworm powder at a dose of 200 µl inhibits the bacterial growth (Table 3). This indicated that EWP at a dose of 200 µl is a minimum concentration to inhibit the growth of the selected bacteria.

The observed antibacterial activity is attributed to the presence of bioactive compounds in the extracts of earthworm powder tested. The presence of these bioactive compounds in extracts is known to confer antibacterial activity against disease-causing microorganisms. Antimicrobial agents of earthworms digestive fluid are formed in the earthworm body but not by the soil microorganism entering their digestive tract (Khomyakov et al.,2007) They have observed that the digestive fluid of earthworm show the same antimicrobial activity after feeding on soil and sterile sand, and partial sterilization of the gut with streptomycin does not lower the antimicrobial activity. Antimicrobial activity in the guts of earthworms derived from metabolites of symbiotic bacteria from the gut walls is possible. The antimicrobial activity of *Eisenia foetida* coelomic fluid directed against Gram-positive and negative bacteria was analyzed. The gut extracts of earthworms have antibacterial and antifungal activity (Shobha and Kale, 2008). The new bacterial strain with antimycobacterial activity has been isolated from the midgut of *Dendrobaena veneta* (Annelida) (Marta et al., 2010). Earthworm species have rich diversity. Based on their living environments, it is rational to think that there are effective anti-infective agents in earthworm's skin.The dried earthworm powder of *P.excavatus* shows more activity(60 µg/mL/disc) than the *L.mauritii* (Prakash and Gunasekaran,2011). Cho et al. (1998) identified the first antimicrobial peptide (lumbricin I) from the earthworm, *Lumbricus rubellus*. Lumbricin I is considered as a proline-rich antimicrobial

Table.1 Determination of antibacterial activity by disc diffusion method

Microorganisms	Diameter of zone of inhibition (mm)			
	Petroleum ether extract	Ethanolic extract	Aqueous extract	Erythromycin control
Staphylococcus aureus	**12**	**15**	**10**	**18**
Salmonella typhi	**10**	**10**	**4**	**12**
Aeromonas hydrophila	**8**	**16**	**8**	**18**

Table.2 Determination of antifungal activity by disc diffusion method

Microorganisms	Diameter of zone of inhibition (mm)			
	Petroleum Ether extract	Ethanolic extract	Aqueous extract	Erythromycin control
Aspergillus niger	**6**	**9**	**8**	**10**
Candida albicans	**7**	**12**	**11**	**12**

Table.3 Antibacterial activity (MIC) tested for the crude earthworm powder using optical density method

Bacterial culture	Concentration of Ethanolic extract of earthworm powder	OD Value
Staphylococcus aureus	Control	24.57 ± 0.91
	50µl	15.25 ± 2.01
	100 µl	13.66 ± 0.33
	150 µl	8.72 ± 1.15
	200 µl	6.76 ± 1.14
Salmonella typhi	Control	15.00 ± 0.57
	50µl	13.33 ± 0.66
	100 µl	9.33 ± 0.33
	150 µl	7.66 ± 0.33
	200 µl	7.33 ± 0.66
Aeromonas hydrophila	Control	37.05 ± 1.50
	50µl	27.01 ± 1.29
	100 µl	17.6 ± 0.3
	150 µl	11.4 ± 0.66
	200 µl	3.71 ± 1.54

peptide containing 62 amino acids including proline (15%) with a molecular weight of 7231 Da. Lumbricin I showed antimicrobial activity *in vitro* against a broad spectrum of microorganisms without hemolytic activity . Recently, two antimicrobial peptides (PP1 and OEP3121) have been identified from earthworms of *Pheretima tschiliensis* and *E. foetida*, respectively (Wang et al., 2003). Engelmann et al. (2004) and Balamurugan et al. (2008) found that

earthworm coelomic fluid contain biologically active molecules and leukocytes that participate in phagocytosis, encapsulation and killing of HeLa, HEp-2, PC-12 and PA317 cells *in vitro*. Presumably, earthworms synthesize and secrete several effective modulators of innate immune responses such as antibacterial molecules, cytotoxic proteins and cytokines.

The present studies report that vermicompost is rich in microbial populations and diversity, particularly fungi and bacteria. It is possible that earthworms can be used not only in environmental monitoring but also in the acquisition of novel molecules for human therapeutic purposes. This study clearly indicates that the earthworm powder lead to formulation of new natural antimicrobial agent and thus may found beneficial in future prospects for mankind. Thus solvent extracts of earthworm can be used against serious and dreadful pathogenic microorganisms responsible for causing serious pathogenic disorders.

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References

Balamurugan, M., K .Parthasarathi, E.L. Cooper and Ranganathan ,L.S. 2008. Anti-inflammatory and anti-pyretic activities of biologically active extract isolated from whole earthworm, *Lampito mauritii*. J. Ethnopharmacol. 121(2):330-2.

Cho, J.H., C.B. Park, Y.G. Yoon and Kim, S.C.1998. Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization. Biochim. Biophys. Acta. 1408:67-76.

Cooper, E.L., B. Ru and Weng, N. 2004 Earthworms: sources of antimicrobial and anticancer molecules. Advances in Complementary and Alternative Approaches to Biomedicine, Kluwer Academic/Plenum, New York, NY, USA, 359-390.

Engelmann, P., J. Kiss, E.L. Cooper and Nemeth, P. 2004. Earthworm leukocytes kill HeLa, HEp-2, PC-12 and PA317 cells in vitro. J.Biochem. Biophys. Methods. 61:215-228.

Hong, C., 2007. Earthworm fibrinolytic enzyme and anti- tumor activity on human hepatoma cells, *in vitro* and *in vivo*. Chinese Med. J. 260-264.

Khomyakov ,N.V., S.A. Kharin, Nechitailo, TYu, P.N. Golyshin, A.V. Kurakov, B.A. Byzov and Zvyagintsev, D.G. 2007. Reaction of microorganisms to the digestive fluid of earthworms. Microbiology. 76:45-54.

Kim, Y.S., M.K. Pyo, K.M. Park, B.S. Hann, K.Y. Yang and Yun-Choi, H.S. 1998. Dose dependency of earthworm powder on antithrombotic and fibrinolytic effects. Arch. Phamacal Res., 21: 374-377.

Marta, J., P .Fiołka Mirosław, D. Zagaja Tomasz, Piersiak Marek Wróbel, Jarosław Pawelec. 2010. Gut bacterium of *Dendrobaena veneta* (Annelida: Oligochaeta) possesses antimycobacterial activity. J. Invertebr. Pathol. 105:63-73.

Mihara, H., R. Ikeda and Yonnet, T. 1996. The Useful of Earthworm Powder. Miyazaki Medical College, Kiyotake, Miyazaki.

Prakash, M., and Gunasekaran, G. 2011. Antibacterial Activity of the Indigenous Earthworms *Lampito mauritii* (Kinberg) and *Perionyx excavatus* (Perrier).The J. Alterna. and Complemen. Med. 17(2): 167-170.

Rios, J.L., M.C. Recio and Villar, A. 1988.Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol. 123:127-129.

Shobha, S.V., and Kale, R. 2007. Antimicrobial potency of earthworm, *Eudrilus eugeniae* on certain plant pathogens. Administrator .

Shobha ,S.V., and Kale, R.D .2008. In *vitro* studies on control of soil-borne plant pathogens by earthworm *Eudrilus eugeniae* exudates. Green Pages. http://www.ecoweb.com/ editorial/ 080106.html.

Wang, X., X.X. Wang and Zhang, Y.2003. An antimicrobial peptide of the earthworm Pheretima tschiliensis: cDNA cloning, expression and immunolocalization. Biotechnol. Lett. 25:1317-1323.

Yegnanarayan , R., P.P. Sethi, P.A. Rajhan, K .Pulandiran and Ismail ,S.A. 1987. Anti-inflammatory activity of total earthworm extracts in rats. Indian J. Pharmacol. 19:221-224.