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INVITRO ANTIBACTERIAL ACTIVITY OF *EISENIA FOETIDA* MUCUS EXTRACT AGAINST CLINICAL PATHOGENS OF HUMAN BEINGS

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Abstract:

Earthworms are macro invertebrate and have been widely used as therapeutic drugs for thousands of years. In the current research, experiments viz., the antibacterial activity of mucus extracts of Eisenia foetida were conducted to investigate for the first time in Tamilnadu against human infectious pathogens. Antibacterial activity of E. foetida against human pathogens underwent investigation through an agar disc diffusion method. The percentage of bacterial growth was analyzed statistically with One-Way Analysis of Variance (ANOVA). Results showed that the mucus of E. foetida produced a strong potent antibacterial. Bacillus subtilis exhibited the highest inhibition zone (23.37±1.93 mm), followed by Echerichia coli (20.35±1.35mm), Klebsiella pneumonia (18±0.21), Staphylococcus aureus (17.3±1.4 mm), Staphylococcus epidermidis (15.63±0.98 mm), Streptococcus pyogenes (14.77±1.03 mm). The results clearly indicate that the mucus and solvent extracts contain effective antimicrobial properties and bioactive compounds to inhibit the growth of infectious pathogens. We conclude that mucus extracts of earthworm have significant level of antimicrobial activities and in future could be potentially used against various infectious pathogens.

Key words: *E. fortida. Earthworm, antibacterial activity, disc diffusion method.*

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INTRODUCTION:

Earthworms represent the largest members of Oligocheata (Phylum *Annelida*) and soil lodging invertebrates. The worms reside worldwide and live in water, soil and manure containing abundant microorganisms that are ingested during feeding [2]. Oriental medicine recognized earthworms as anti-inflammatory, analgesic and antipyretic agents [3]. The earthworms also contain anti-coagulatant or fibrinolytic activity, which facilitates the thinning of blood [4]. The literature also describes the medicinal properties of earthworms. Researchers studied the antimicrobial potency of earthworm (*Eudrilus eugeniae*) extracts on certain plant pathogens (Shobha and Kale, 2008). Researchers also used a paste of minced earthworm (*Lampito mauritii*, Kinberg) to reduce oxidative, inflammatory, serum biochemical and haematological indices in inflamed rat [1]. The extracts of *Pheretima hawayana* (Rosa) and *Allolobophora caliginosa* (Savigny) also showed antioxidant, anti-inflammatory, and antipyretic activity [3]. Although, researchers frequently study earthworms all over the world, investigators in india have seriously neglected this area of research.

Earthworms have been used in medicine for various remedies since 1340 AD. Earth-worm has been recognized in oriental medicine as anti-inflammatory, analgesic and antipyretic agent [5]. It shows anticancer effect by preventing excess glucose uptake [2]. Microorganisms are known to play a major role in soil characteristics, invertebrates are believed to act as regulators of antimicrobial activity. Earthworms live in an environment filled with various kinds of pathogens. Physiologically and evolutionally speaking, earthworm survival in such an environment must have favoured the development of efficient defense mechanisms against various environmental pathogens during the course of evolution, including the production of certain anti-microbiological substances, especially active proteins and enzymes [6]. Earthworm surface excreta were found to have potent antimicrobial activity. It is also having anti-coagulatory or fibrinolytic activity which results in the facilitation of blood circulation [7]. The earthworm has been sus-pected to contain proteases which dissolve the fibrin clots or anticoagulants which selectively interfere with the intrinsic pathway of blood coagulation cascade. Medicinal properties of earthworm had also been described [3]. Hence, in the present study, the mucus prepared from the earth worm, *E. fotida* was tested for antibacterial potential.

MATERIALS AND METHODS:

Earthworms

Earthworms *Eisenia fetida* taken were from Periyar Maniammai University, Vallam, Thanjavur, Tamilnadu, India The earthworms were identified morphologically using an identification key (Lee 1985).

Preparation of earthworm mucus extract

The *E. fotida* were washed with running tap water and then fed with wet blotting paper for 18 to 20 h to clear their gut. The gut cleared worms were again washed with distilled water. The worms were kept in plastic troughs, covered tightly with polythene cover, and exposed to sunlight for 3 days to kill them. Mucus and coelomic fluid that oozed out digested the dead worms forming a brown coloured paste earthworm mucus [3].The earthworm paste were filtered and the filtrates obtained were condensed in water-bath at 35°C. The crude paste obtained was diluted in 10% DMSO for evaluation of antimicrobial activity.

Test organisms

Bacillus subtilis, *Echerichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* were used in this study. These strains were collected from the Microbiology Laboratory of Medical College, Annamalai University, Chidambaram Tamilnadu, India.

Antibacterial assay

Commercially available standard antibiotics were used for comparison of the antibacterial activity [2]. *In vitro* antibacterial activity of the test samples were assayed according to Barry (1980). Sterile paper disks of 5 mm diameter were impregnated with mixtures of different crude extracts (aqueous, methanol, chloroform, benzene) and then air dried. Each disk contains approximately 0.5µg/ml of crude extract mixture. All disks were stored at 4 °C when not in use. These paper discs were placed on nutrient agar (HiMedia Laboratories, India) inoculated with the test bacteria and incubated at 37 °C for 24 h. Gentamycin (30 µg/disc) (Invitrogen, USA) were used as positive control and blank discs (impregnated with solvents followed by evaporation) were used as negative control. After incubation the culture plates were examined and the zones of inhibition were measured in millimeter scale.

RESULTS:

The antibacterial activity of specific concentrations of earthworm mucus extract of E. fotida is given in Table 1. Results showed that the mucus of *E. foetida* produced a strong potent antibacterial. *Bacillus subtilis* exhibited the highest inhibition zone

(23.37±1.93 mm), followed by *Echerichia coli* (20.35±1.35mm), *Klebsiella pneumonia* (18±0.21), *Staphylococcus aureus* (17.3±1.4 mm), *Staphylococcus epidermidis* (15.63±0.98 mm), *Streptococcus pyogenes* (14.77±1.03 mm). All bacterial species showed resistance against E. fotida mucus extract. MIC was tested at concentration 0.5µg/ml.

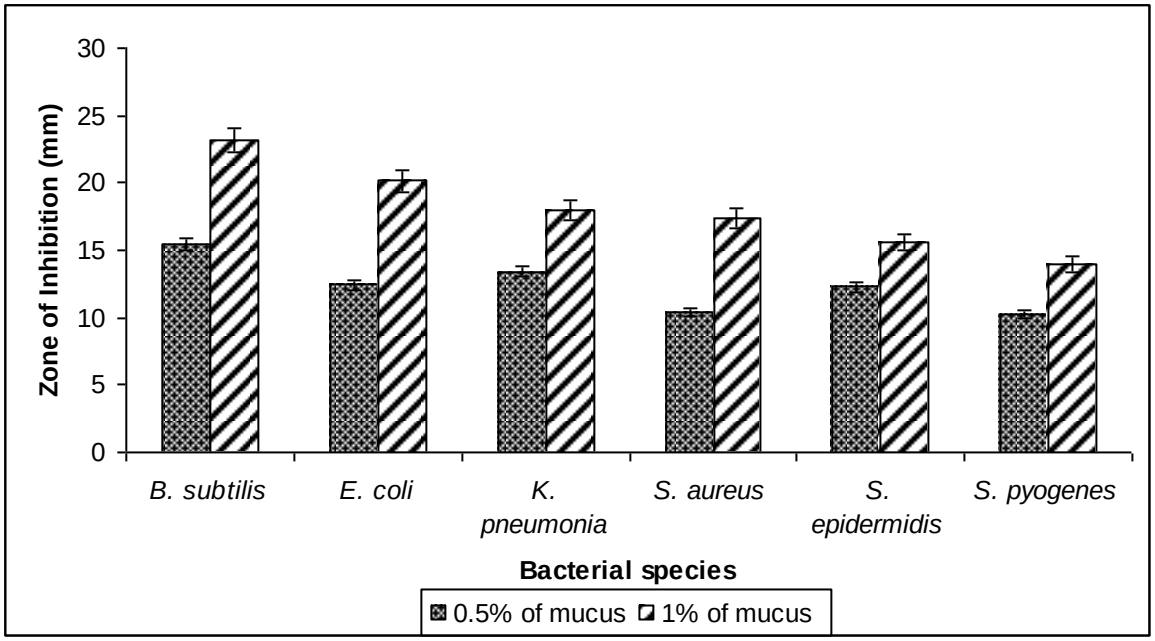

Fig 1: Effect of dE. Fotida mucus extract on clinical bacterial pathogens of human beings.

Table 1: Effect of different solvent of *S. trilobata* extract on clinical bacterial pathogens of human beings.

S. No	Bcaterial species	Zone of inhibition (mm)	
		0.5% of mucus	1% of mucus
1	*B. subtilis*	15.4±0.42	23.13±1.93
2	*E. coli*	12.43±1.43	20.13±1.35
3	*K. pneumonia*	13.43±1.50	18±0.21
4	*S. aureus*	10.43±0.52	17.4±1.4
5	*S. epidermidis*	12.3±1.73	15.6±0.98
6	*S. pyogenes*	10.3±0.68	14±1.03

DISCUSSION:

The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. However, there has been a parallel – and alarming – increase in bacterial resistance to existing chemotherapeutic agents as a result of their injudicious use. In addition, antibiotics are occasionally associated with adverse effects to the host, including hypersensitivity, immune-suppression and allergic reactions.

Khomyakov et al. [11] suggested that antimicrobial agents of earthworms digestive fluid are formed in the earthworm body but not by the soil microorganisms entering their digestive tract. 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. Mendez et al. [13] observed that the guts of earthworms, *Onychochaeta borincana* in sterile soil contain the same microorganisms as the guts of individuals that have not been submitted to the cleansing treatment. 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. Cho et al. [9] identified the first antimicrobial peptide (lumbricin I) from the earthworm, *Lumbricus rubellus*. Lumbricin I is considered as a proline-rich antimicrobial 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 [15]. Recently, another two antimicrobial peptides (PP1 and OEP3121) have been identified from earthworms of *Pheretima tschiliensis* and *E. foetida*, respectively [17]. The antimicrobial peptide, lumbricin-PG was identified from skin secretions of the earthworm, *Pheretima guillelmi* [19]. Engelmann et al. [20] and Balamurugan et al. (2008) found that earthworm coelomic fluid contains 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.

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