SHORT COMMUNICATION

In vitro screening of mucus and solvent extracts of *Eisenia foetida* against human bacterial and fungal pathogens

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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, antifungal and antioxidant activity of mucus and solvent extracts of *Eisenia foetida* were conducted to investigate for the first time in Pakistan against human infectious pathogens. Antimicrobial activity of *E. foetida* against human pathogens underwent investigation through an agar disc diffusion method while an $ABTS^{\cdot+}$ free radical scavenging method assessed the antioxidant activity. The percentage of bacterial and fungal growth was analyzed statistically with One-Way Analysis of Variance (ANOVA). Results showed that the mucus IV of *E. foetida* produced a strong potent antibacterial and antifungal activity. *Pseudomonas aeruginosa* exhibited the highest inhibition zone (33.67±1.53 mm), followed by *Klebsiella pneumonia* (30.33±1.53mm), *Penicillium notatum* (30±0.051), *Escherichia coli* (29±1 mm), *Candida albicans* (28.33±0.54 mm), *Staphylococcus aureus* (27±1mm), *Serratia marcescens* (25.33±0.58 mm), *Aspergillus flavus* (25.33±0.58 mm), *Staphylococcus epidermidis* (24.33±0.58 mm), *Streptococcus pyogenes* (21.67±1.53 mm), and *Aspergillus niger* (20.67±0.53 mm). Mucus IV of *E. foetida* also showed the highest antioxidant activity (99%). 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 and antioxidant activities and in future could be potentially used against various infectious pathogens.

Keywords: *Eisenia foetida*, bacterial pathogens**,** mucus extract, antioxidant activity, antimicrobial activity.

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 (Tasiemski, 2008). Oriental medicine recognized earthworms as anti-inflammatory, analgesic and antipyretic agents (Noda *et al*., 1992). The earthworms also contain anti-coagulatant or fibrinolytic activity, which facilitates the thinning of blood (Wang *et al*., 1989). The literature also describes the medicinal properties of earthworms (Shobha and Kale, 2006). 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 (Balamurugan *et al*., 2009). The extracts of *Pheretima hawayana* (Rosa) and *Allolobophora caliginosa* (Savigny) also showed antioxidant, anti-inflammatory, and antipyretic activity (Omar *et al*., 2012). Although, researchers frequently study earthworms all over the world, investigators in Pakistan have seriously neglected this area of research. Little information is available in Pakistan on the indigenous fauna of worms regarding their utility in vermicomposting or in improving soil health. The regions of Azad Jammu and Kashmir, Pakistan have not conducted studies on the biodiversity, anti-microbial or anti-oxidant activities of earthworm. These regions will conduct research on these properties of earthworms for the first time. Researchers recently collected and studied earthworms from the different areas of Azad Jammu and Kashmir using traditional methods. China and other parts of the world used earthworms for many centuries as a source of therapeutic drugs for a variety of diseases (Ismail, 2005). Microorganisms participate in the ecology of soil characteristics and invertebrates act as antimicrobial activity regulators (Mathur *et al*., 2009). Earthworms respond to microbial infection through discharging their skin secretions and antimicrobial protein. Prakash and Gunasekaran, (2011) showed the antibacterial activity of *Lampito mauritii* and *Perionyx excavates* extracts against *Staphylococcus epidermidis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

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In the present research, exposing the earthworms to various solvents and extracted the excretions for study purposes. Using the agar disc diffusion methods, the extracted excretions acted as antimicrobial agent against various Gram negative, Gram-positive bacterial and fungal pathogens. This represents new research done in Pakistan to use the earthworm extracts against pathogenic microorganisms responsible for causing serious and deadly diseases.

MATERIALS AND METHODS

Ethics statement

All experiments have been designed to avoid distress, unnecessary pain and suffering to the experimental animals. All experimental procedures were conducted in accordance with international guidelines (Matthiessen *et al*., 2003) and regulation referred as Wet op de dierproeven (Article 9) of Dutch Law.

Sampling and identification of earthworm

Earthworms were collected from different localities of Muzaffarabad, Azad Jammu and Kashmir, Pakistan, and transported the specimens to Biotechnology laboratory, Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. The specimens were rinsed with running tap water to remove the sand particles. Following the washing procedure, a digital camera (Canon, EOS 350D, EF-S18-55, Kit, Japan) was used to photographed the earthworms. Earthworms were identified on the basis of morphological features such as color, number of segments, prostomium and clitellium shape, and segments under binocular microscope. Further identification of earthworms were carried out by two international known experts, Lusty Istiqomah (Researcher at Division of Feed and Animal Nutrition, Indonesian Institute of Sciences (LIPI), Indonesia) and Jorge Domı´nguez (Department of ecology and biology of Animal, University of deVigo, E-36200 Vigo, Spain).

Preparation of powder

Some of the earthworms were then placed on sterile Petri dishes and dried in an incubator for 48 h at 55°C. After incubation the earthworms were removed and crushed into a fine powder. This powder was stored at room temperature before analysis.

Preparation of extracts of Eisenia foetida

For extract preparation, twenty grams of earthworm powder were homogenized with different solvents *viz*., ethanol, methanol, chloroform and diethyl ether, to increase-polarity for two weeks. On the other hand healthy earthworms were placed in water for several days, and skin secretions (mucus) of earthworm were collected. The four groups of mucus specified as; Mucus I indicated the extract of earthworm collected after 3 days, Mucus II after 6 days, Mucus III after 9 days and Mucus IV after 12 days (fig. 1). The homogenized mixtures in different solvents underwent collection, filtration and evaluation for antimicrobial and antioxidant activity.

Isolation of test bacterial and fungal pathogens

Bacterial and fungal pathogens such as *Klebsiella pneumonia, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aureginosa, Serratia marcescens, Escherichia coli, Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, and *Penicillium notatum* were isolated from infected human samples like urine, pus and blood from individuals of different ages and genders from the Microbiology and Pathology Lab of Combined Military Hospital (CMH) Muzaffarabad, Azad Jammu and Kashmir, Pakistan.

Estimation of trace elements

Nitric acid and sulphuric acid digestion methods were used for the estimation of various trace elements including Magnesium (Mg), Calcium (Ca), Iron (Fe), Phosphorous (P), Manganese (Mn), Zinc (Zn), Copper (Cu), nitrogen (N) and potassium (K) in the earthworm powder (Homer, 2003).

ABTS decolorization assay of Eisenia foetida extracts

ABTS·+ scavenging activity to evaluate the antioxidant potential of earthworm extracts according to the described method of Re *et al*. (1999). The ABTS stock solution was prepared by reacting potassium persulphate (2.45mM) and ABTS (7mM), then allowing the mixture to stand for at least 16 h to generate $ABTS^+$ free radicals. The running solution was organized by diluting the stock solution with various solvents and the absorbance of solutions were recorded at 734nm ($Ao_{Control}$). For tests, 1ml of ABTS running solution was merged with 10µl extracts of different solvents and mucous (0-100µg/ml). The absorbance of test samples (Ai_{Sample}) was also observed at 734 nm precisely 10 min after the reaction mixture was ready. In both attempts, the drug, quercetin, was used as a positive control. The percentage radical scavenging activity (% RSC) was calculated using the formula: $\% RSC = [(Ao_{Control} - Ai_{Sample}) / Ao_{Control}] \times 100\%$

Antibacterial activity of Eisenia foetida extracts

In vitro agar disc diffusion method determined the antibacterial activity of various extracts of earthworm (Luangtongkum *et al*., 2007; Jorgensen and Turnidge, 2007; Gaudreau *et al*., 2008). Nutrient agar and Nutrient Broth Medium (NAM; Oxide CMOO3 and NBM; CM1) provided the mediums for bacterial growth. The overnight culture was mixed with freshly prepared nutrient agar medium at 45°C and poured into the sterilize Petri dishes. Laminar flow housed all Petri dishes at room temperature for solidification. The 5mm discs were soaked with various prepared extracts of earthworm and placed on agar surface. The Petri dishes underwent incubation at 37°C for 48h. Discs of chloroform, ethanol, methanol and diethyl ether were used as negative controls. Microbial

growth was assessed by calculating the zone of inhibited diameter (mm) after 48h. Before each experiment, spectrophotometry measured the optimal density (OD) of bacterial growth of 10^7 colony forming units (cfu)/ml at the wavelength of 600 nm (Seeley *et al*., 2001). Researchers performed each culture procedure in triplicate. The results of the sensitivity tests were expressed as (0) for no sensitivity, (below 10) for low sensitivity, (11-20) for moderate sensitivity and (21-35) for high sensitivity.

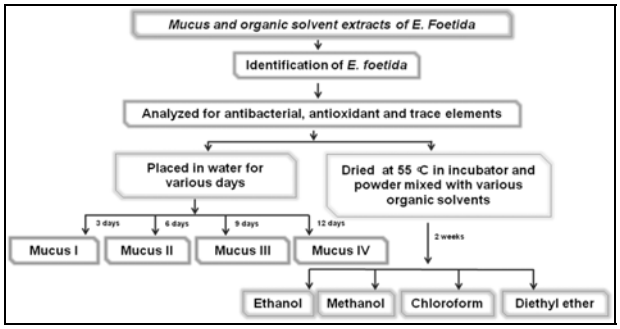

Fig. 1: Flow diagram of preparation of various extracts of earthworm.

Fig. 2: Morphological features of *E. foetida*. (A) Healthy live earthworm, (B) Preserved in 40% formalin for morphological studies.

Antifungal activity of Eisenia foetida extracts

An agar disc diffusion method was also performed to detect antifungal activity of various extracts of earthworm (Luangtongkum *et al*., 2007; Jorgensen and Turnidge, 2007; Gaudreau *et al*., 2008). The microorganisms were grown on Sabouraud dextrose broth (SADB: Hi-media) for 24h at 25^0C. Spectrophotometry measured the optimal density (OD) of fungal growth 10^8cfu/ml at the wavelength of 530nm. The drug, Nystatin (30µg), was used as a positive control. Each treatment was completed in triplicate and sensitivity tests results expressed as (0) for no sensitivity, (below 10) for low sensitivity, (11-20) for moderate sensitivity and (21-35) for high sensitivity. Researchers calculated the percentage of inhibition for fungal pathogen as described by Abd-Ellatif *et al*. (2011): % inhibition= 100- (growth inhibited by test/growth inhibited by control×100).

Antibiotics sensitivity test

The antibiotics for positive control consisted of Amoxicillin (10µg), Penicillin G (10µg), Ampicillin (10 µg) and Oxytetracycline (10µg) and were active against *K. pneumonia, S. epidermidis, S. aureus, S. pyogenes, P. aureginosa, S. marcescens* and *E. coli*. Researchers assessed the sensitivity of antibiotics against test strains by the agar disc diffusion method (Luangtongkum *et al*., 2007; Jorgensen and Turnidge, 2007; Gaudreau *et al*., 2008).

STATISTICAL ANALYSIS

Each experiment was repeated three times and expressed the Mean ± Standard Deviation (M±SD) from absolute data using an on-line Standard Deviation calculator (http://easycalculation.com/statistics/standard-deviation.php). The percentage of bacterial and fungal growth was analyzed statistically with ANOVA found at the website http://www.danielsoper.com/statcalc3/calc.aspx?id=43 to distinguish differences between the means (Gomez and Gomez, 2007). The evaluation of antibacterial and antifungal activity of the solvent and mucous extracts of *E. foetida* with standard antibiotics was also confirmed using activity index (AI) (Shekhawat and Vijayvergia, 2010). The level of significance was set at $p < 0.05$ and $p < 0.1$.

RESULTS

Identification of Eisenia foetida

The earthworms were collected from the local soil of Muzaffarabad city of Azad Jammu and Kashmir, Pakistan and identified by the experts in Indonesia and Spain. *E. fetida* lives on the surface of the soil as epigeic worms. Some external features were recorded on the basis of color, number of segments, prostomium shape, clitellium shape and segments. *E. fetida* is (35-130mm) in length, 3-5mm in diameter, clitellum over segments either 24, 25, 26-32, 80-120 segments, first dorsal pore between 4/5 or 5/6 segments, seminal vesicles, four pairs on in 9-12 and spermatheca, two pairs in 9/10 and 10/1 (fig. 2A and 2B). Earthworms come in various colors. The dorsal side of the worm is darker whereas the ventral side appears paler in color. The colors range from brownish black to purple with the most common color being reddish brown due to presence of hemoglobin in the blood (fig. 2A and 2B). *E. fetida* may also be known as Tiger worm, Red worm, Manure worm, and Red Wiggler. The optimum growing temperature for *E. fetida* is (20° to 25°C) but they can tolerate a range between 4° to 27°C. On the other hand *E.*

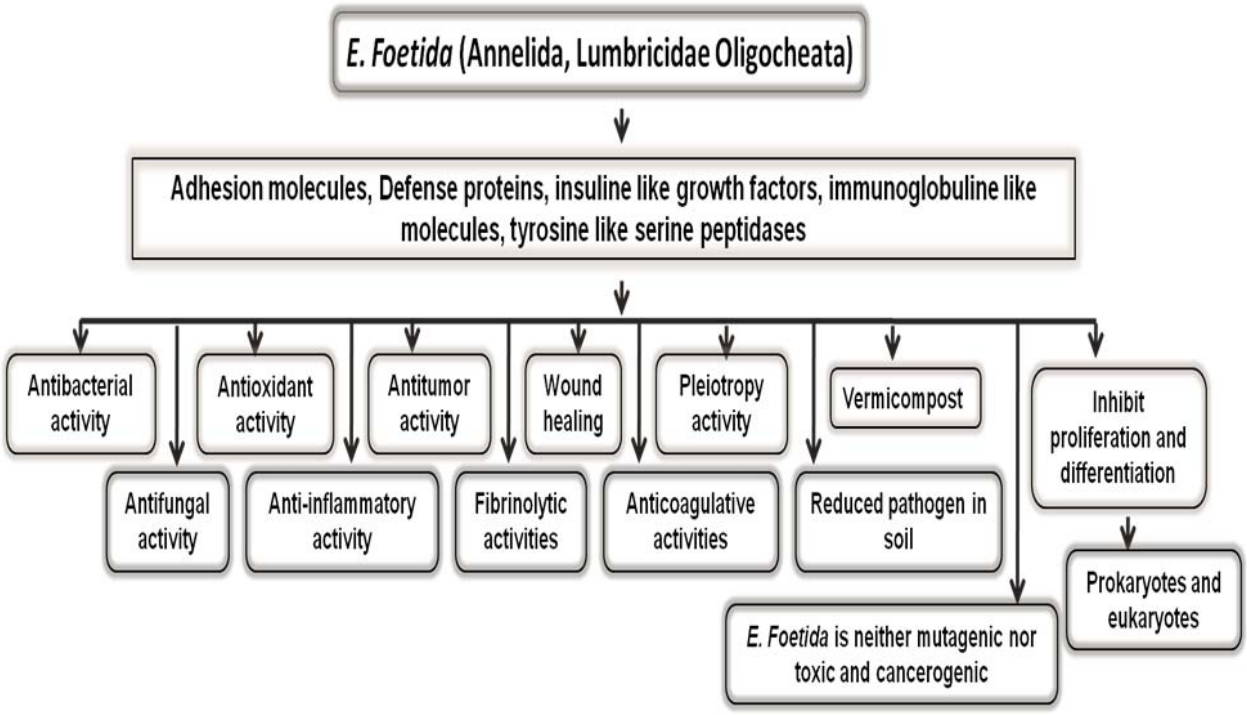

Fig. 3: Applications of extracts of *E. foetida***.**

fetida become overheated at 85°F and can quickly die at above 90°F (Domínguez, 2004).

Trace elements of Eisenia foetida

Iron appears as the trace element with the highest concentration in dried *Eisenia fetida* (280 mg/l) while magnesium exists as the lowest concentration at 0.046 mg/l. The trace element concentrations ranked from highest to lowest are iron, zinc, manganese, cupper, nitrogen, phosphorous, potassium, calcium and magnesium, respectively.

Sensitivity analysis

Results showed that *E. coli* and *S. pyogenes* demonstrated resistance against all tested antibiotics. Amoxicillin showed zones of inhibition against *K. pneumonia*, *S. epidermidis, S. aureus, S. marcescens,* and *P. aeruginosa* (6, 2, 6, 2 and 6 mm). Likewise, Ampicillin showed inhibitory effects against *K. pneumonia, S. aureus* and *S. marcescens* (6, 6 and 8 mm) and resistant against *S. epidermidis, P. aeruginosa, E. coli* and *S. pyogene*. Oxytetracycline exhibited an inhibition zone of 8, 5, 6 and 6 mm for *K. pneumonia, S. epidermidis* and *P. aeruginosa,* respectively. Penicillin G showed inhibition zones of 8 and 6 mm against *S. marcescens* and *P. aeruginosa.*

Antioxidant activity of Eisenia foetida extracts

Mucus IV extract of *E. foetida* showed the highest antioxidant activity (99%) of all the extracts, followed in descending order by Mucus III (96%), Mucus II (74%), Mucus I (72%), ethanol (70%), methanol (39%), chloroform (30%) and diethyl ether (12%).

Antibacterial activity of Eisenia foetida extracts

In this current research, the agar disc diffusion method measured the antibacterial activity of organic solvent extracts and mucus excreta of *E. foetida*. All the mucus excreta and organic solvent extracts of *E. foetida* showed varying degrees of antibacterial and antifungal activities against the sample of human bacterial and fungal pathogens (table 1 and 2). Similarly, the mucus was found to be more effective than organic solvent extracts against all pathogens (table 1 and 2). Mucus extracts showed maximum antibacterial activity in comparison to solvent extracts against *S. epidermidis, S. pyogenes, S. aureus, S. marcescens, P. aeruginosa* (table 1). The highest zone of inhibition of mucus IV was measured against all the samples of human pathogens. *Pseudomonas aeruginosa* exhibited the highest inhibition zone (33.67±1.53 mm), followed by *K. pneumonia* (30.33±1.53 mm), *E. coli* (29±1 mm), *S. aureus* (27±1 mm), *S. marcescens* (25.33±0.58 mm), *S. epidermidis* (24.33±0.58 mm), and *S. pyogenes* (21.67±1.53 mm), respectively. On the other hand mucus I, mucus II and mucus III demonstrated moderate antibacterial activity with inhibition zones ranging between 12 and 16 mm for the different bacteria tested (table 1). The ANOVA single factor analysis at $P<0.05$, showed that the F value (12.658) is greater than the F critical value (4.001). Therefore a significant difference exists between the mucus and solvent extracts of earthworm inhibiting the growth of bacterial pathogens

(table 3.A and 3.B). In the present study, the organic extracts of *E. foetida* showed less antibacterial activity with inhibition zones ranging between 2 and 10 mm.

Antifungal activity of E. foetida extracts

The current research utilized antifungal assays of mucus and organic solvent extracts of *E. foetida* against four different strains of fungi including *Candida albicans, Aspergillus niger, Aspergillus flavus,* and *Penicillium notatum*. The mucus extracts and Diethyl ether significantly inhibited the fungal strain as compared to the chemicals of chloroform, ethanol and methanol extracts (table 2). The mucus IV showed the highest antifungal potential. Of the four strains tested; mucus IV inhibits the growth of *P. notatum* the most (30±0.051 mm) followed in descending order by *C. albicans* (28.33±0.54 mm), *A. flavus* (25.33±0.58 mm)–and *A. niger* (20.67±0.53 mm). The drug, Nystatin, showed the highest zone of inhibition (23.33±0.33) against *C. albicans*. The ANOVA single factor analysis with $P<0.05$, demonstrated F value (3.034) lesser than the F critical value (4.1709). Therefore, no significant difference exists between the mucus and solvent extracts of earthworm inhibiting the growth of fungal pathogens (table 3.A and 3.B).

DISCUSSION

E. fetida is an epigeic species associated with high organic matter content (Elvira *et al*., 1996). The concentration of elements obtained from dried *E. foetida* contained composition of vermicompost elements as reported previously (Dickerson, 1994; Mihara *et al*., 1996). The literature showed that *E. foetida andrei* coelomic fluid possessed an antibacterial activity against *Bacillus megaterium* and *Aeromonas hydrophila* (Valembios *et al*., 1982). Rivai *et al*, (2003) showed the antibacterial activity of various solvent extracts of *Pontoscolex corethrurus* against *S. aureus* and *E. coli* by measuring inhibition zones. This research group reported on the effective antibacterial activity of n-hexane, chloroform and methanol (Rivai *et al.*, 2003).

Antibacterial activity

Our results consistent with the previous literature showing that surface excreta of the earthworm exhibits potent antimicrobial activity (Oleynik and Byzov, 2008). These results also illustrate the fact that the products of earthworms can decrease the growth of pathogenic bacteria. Consequently, this activity may promote the growth of agriculturally useful bacteria, such as *Rhizobium leguminosarum* or *Azotobacter chroococcum* (Popović *et al*., 1998). The literature previously documented that the antipyretic and anti-inflammatory activities of biologically active extract isolated from *Lampito mauritii* (Balamurugan *et al*., 2009). Other researchers studied the antitumor activities of earthworm fibrinolytic enzyme on human hepatoma cells (Chen *et al*., 2007). Liu *et al,* (2004) demonstrated the antibacterial activity of *E. foetida* from the presence of OEP3121 peptides in the worm. Ansari and Sitaram, (2011) reported that earthworm powder obtained from *E. fetida* possesses antibacterial activity against *P. aeruginosa S. aureus*, and *E. coli*. The earthworm contains peptides coelomycetes that includes lysozyme (Engelmann *et al*., 2005). Li *et al.* (2011) isolated *Lumbricin*-PG from skin secretions of the earthworm *Pheretima guillelmi.* Tasiemski, (2008) and Tasiemski *et al.* (2006) stated that antimicrobial peptide, hedistin, identified from the *Nereis diversicolor* coelomocytes exhibits antibacterial activity against *S. aureus.* Meanwhile, it has been reported that the mucus layer on the skin surface of *E. fetida* consists of several antimicrobial agents that provided a first-line of defense against invading pathogens such as *E. coli* and *S. aureus* consistent with our findings (Wang *et al*., 2011; Wang *et al*., 2011). Pan *et al*. (2003) demonstrated that earthworm *E. fetida* extract had a maximum activity against the Gram-negative strain *P. aeruginosa.* Later, Wang *et al.* (2007) reported antibacterial peptides (ECP) of *E. fetida* extract to *P. aeruginosa.* Ansari and Sitaram (2011) also stated that an extract of *E. fetida* in water (1:1) had antimicrobial activity against *P. aeruginosa*. Mucus extracts of *E. foetida* showed significant results in comparison to the extracts of organic solvents through activity index (table 4). Past observations indicate that *E. foetida* possesses medicinal properties.

Antifungal activity

Previous research studied the antimicrobial potency of *Eudrilus eugeniae* extracts against plant pathogens and found it effective to control the sporulation of fungal pathogens (Shobha and Kale, 2006). Body wall and gut secretions of earthworm exhibit inhibitory effect on *Fusarium oxysporum* (Khalifa, 1965; Shobha and Kale, 2006). Previous investigations on the body wall, gut and coelomic fluid extracts of *E. eugeniae* showed antifungal activity against animal fungal pathogens such as *Candida albicans, Cryptococcus neoformans* and *Trycophytan metagrophyte* (Shobha and Kale, 2006).

The biologically active properties of extracts of various earthworm species were also reported which include antitumor (Chen *et al*., 2007), antioxidant (Omar *et al*., 2012), antibacterial (both facultative and non pathogenic bacteria) (Popovic *et al*., 2005; Shobha and Kale, 2006; Damayanti *et al*., 2008; Damayanti *et al*., 2009; Matausijc-Pisl *et al*., 2010), antifungal (Bogaerts *et al*., 2010), anti inflammatory (Mathur *et al*., 2011; Omar *et al*., 2012), anticoagulative (Prasad *et al*., 2006), and wound healing activities, as well as acting as a vermicompost, reducing pathogens in the soil, inhibiting proliferation of prokaryotes and differentiation in eukaryotes (fig. 3). All of these functional properties arise from the internal presence in the worms of some antibacterial proteins, defense proteins, insulin-like

Table 1: Antibacterial activity of mucous and solvent extracts of *E. foetida* against bacterial pathogens

Bacterial pathogens	Zone of inhibition of mucus and solvent extracts of E. foetida (mm) (M±SD)							
	Group I				Group II			
	Mucus I	Mucus II	Mucus III	Mucus IV	Diethyl ether	Chloroform	Ethanol	Methanol
K. pneumoniae	0	0	0.33±0.58	30.33±1.53	22.33± 19.39	10.33 ±2.52	18±2	17.33± 15.04
S. pyogenes	13±1	10±1	10.33±0.57	21.67±1.53	0	3±1	4±1	3.33±1.53
S. epidermidis	13±2.64	9.66±0.58	13.67±1.53	24.33±0.58	3.67±0.58	6.67±2.52	7.67±0.58	3.67±0.58
S. aureus	13±1	12.67±1.53	13.33±0.58	27±1	5±1	5.67±1.53	4.67±1.53	6.67±0.58
S. marcesscens	14.67±0.58	14±1.73	15.67±0.58	25.33±0.58	3.67±0.58	5.66±0.58	5.66±2.08	5.33±0.58
P. aeruginosa	23.66±8.08	16.33±11.02	10±2.64	33.67±1.53	2.33±4.04	11±2	18.67±4.04	7.33±0.58
E. coli	7.66±0.58	2±0	5±1	29±1	5±1	5.67±1.53	5.66±3.05	8.33±5.13

Table 2: Antifungal activity of mucous and solvent extracts of *E. foetida* against fungal pathogens

Fungal pathogens	Zone of inhibition of Nystatin, mucus and organic solvent extracts of E. foetida (mm) (M±SD)								
	Group I				Group II				Antibiotic
	Mucus I	Mucus II	Mucus III	Mucus IV	Diethyl ether	Chloroform	Ethanol	Methanol	Nystatin (30µl)
Candida albicans	12.33 ±0.33	13.33 ±0.33	17.33 ±0.33	28.33 ±0.54	18.33 ±0.60	10.33 ±0.53	17±0.20	15.33 ±15.04	23.33 ±0.33
Aspergillus niger	11.00 ±0.47	12.00 ±0.57	14.33 ±0.33	20.67 ±0.53	16.67 ±0.33	9.00 ±0.57	9±1	13.33 ±1.53	7.66 ±0.33
Aspergillus flavus	11.33 ±0.23	12.33 ±0.33	17.00 ±0.57	25.33 ±0.58	17.33 ±0.45	13.33 ±0.33	11.67 ±0.58	13.67 ±0.58	8.66± 0.33
Penicillium notatum	15.66 ±0.33	14.66 ±0.33	15.33 ±0.33	30± 0.051	15± 0.021	11.66 ±0.53	15.67 ±1.53	16.67 ±0.58	10.66± 0.33

Growth of inhibition was recorded as (0) for no sensitivity, (below 10) for low sensitivity, (11-20) for moderate sensitivity and (21-35) for high sensitivity M±SD; indicates Mean ± Standard Deviation

Table 3A: Statistical analysis of zone of inhibition against bacterial and fungal pathogens

Pathogens	Groups	Number of subject	Sum	Grand mean	Standard deviation
Bacterial pathogens	Group I	28	409.309	14.6182	9.32234
	Group II	28	206.319	7.36857	5.41747
Fungal pathogens	Group I	16	270.960	16.935	6.04259
	Group II	16	223.988	13.9993	2.98924

Table 3B: One way ANOVA of zone of inhibition against bacterial and fungal pathogens

ANOVA analysis of mucus and solvent extracts of E. foetida against bacterial pathogens						
Source of variation	SS	Df	MS	F value	P value	F crit
Between groups	735.800	1	735.800	12.658	0.001*	4.001
Within groups	3,138.885	54	58.128			
Total	3,874.685	55				
ANOVA analysis of mucus and solvent extracts of E. foetida against fungal pathogens						
Source of variation	SS	Df	MS	F value	P value	F crit
Between groups	68.947	1	68.947	3.034	0.0092**	2.880
Within groups	681.727	30	22.724			
Total	750.673	31				

*P<0.05; **P<0.1

growth factors, immunoglobulin-like molecules and tyrosine-like serine peptidases (fig. 3: Popović *et al*., 1988). Other researchers studied fibrolytic enzyme activity of earthworm in both *in vivo* and *in vitro* systems (Chen *et al.,* 2007).

Table 4: Activity index (AI) of mucous and solvent extracts of *E. foetida* against bacterial pathogens

Pathogens	AI= Zone of inhibition of mucus or solvent extracts / Zone of inhibition of antibiotic								Mean values of Zone of inhibition of Antibiotics (mm)
	Group I				Group II				
	Mucus I	Mucus II	Mucus III	Mucus IV	Diethyl ether	Chloroform	Ethanol	Methanol	
K. pneumoniae	A>E	A>E	A>E	5.05	3.7	1.7	3	2.8	Amoxicillin (6)
	♣	♣	♣	E>A	E>A	E>A	E>A	E>A	Pencilline G (0)
	A>E	A>E	A>E	5.05	3.7	1.7	3	2.8	Ampicillin (6)
	A>E	A>E	A>E	3.78	2.78	1.28	2.25	2.16	Oxytetracycline (8)
S. pyogenes	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Amoxicillin (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Pencilline G (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Oxytetracycline (0)
S. epidermidis	6.5	4.85	6.85	12.15	1.85	3.35	3.85	1.85	Amoxicillin (2)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Pencilline G (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
	2.6	1.94	2.74	4.86	0.74	1.34	1.54	0.74	Oxytetracycline (5)
S. aureus	2.16	2.11	2.21	0.83	1.11	0.95	0.78	4.50	Amoxicillin (6)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Pencilline G (0)
	2.16	2.11	2.21	4.5	0.83	0.95	0.78	1.11	Ampicillin (6)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Oxytetracycline (0)
S. marcescens	7.15	7	7.85	12.65	1.65	2.85	2.85	2.65	Amoxicillin (2)
	1.78	1.75	1.96	3.16	0.41	0.71	0.71	0.66	Pencilline G (8)
	1.78	1.75	1.96	3.16	0.41	0.71	0.71	0.66	Ampicillin (8)
	2.38	2.33	2.61	4.21	0.55	0.95	0.95	0.88	Oxytetracycline (6)
P. aeruginosa	3.95	2.7	1.67	5.61	0.38	1.83	3.11	1.21	Amoxicillin (6)
	3.95	2.7	1.67	5.61	0.38	1.83	3.11	1.21	Pencilline G (6)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
	3.95	2.7	1.67	5.61	0.38	1.83	3.11	1.21	Oxytetracycline (6)
E. coli	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Amoxicillin (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Pencilline G (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Ampicillin (0)
	E>A	E>A	E>A	E>A	E>A	E>A	E>A	E>A	Oxytetracycline (0)

E>A & >1 activity index values indicate mucous and solvent extracts of earthworm has higher effect against bacterial pathogens compared to antibiotics; A>E & <1 activity index values indicate antibiotics has higher effect against bacterial pathogens compared to mucous and solvent extracts of earthworm; 1 indicates both have equal effect; ♣ indicates both have no effect.

CONCLUSIONS

This research only investigates the presence of significant antibacterial, antifungal and antioxidant properties of mucus and solvent extracts from the *E. foetida* earthworm. We conclude that mucus extracts of earthworm have significant level of antimicrobial and antioxidant activities and in future could potentially be used against various infectious pathogens.

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