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An Investigation into the Anti-microbial and Anti-fungal Properties of Earthworm Powder Obtained from *Eisenia fetida*

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ABSTRACT

Present study was carried out during the year 2006-2007 on dried earthworm powder collected from culture of *Eisenia fetida* in vermicomposting units and focused on the effect of the dried earthworm on microbes determining the anti-microbial and anti-fungal properties of the worms, as well as the chemical composition of worms obtain from vermicomposting units. The earthworm powder was also subjected to analysis of nitrogen and potassium using standard procedures. Antimicrobial disc diffusion suspecting tests were carried out against Bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) as well as the fungus *Candida albicans* with the following treatments: Control, earthworm powder in water (1:1), earthworm powder in water (1:2) and earthworm powder in acetone (1:2). The technique involves using disk diffusion susceptibility testing where disks from both the pure and diluted honey as well as the antibiotic disk erythromycin (control) were impregnated onto the surface of the Mueller Hinton agar. The study conclusively proved that the earthworm powder (all dilutions) has antifungal properties which would be effective in treating fungal infections, such as candidosis whereas did not indicate the anti-microbial properties which may be attributed to different composition of elements in earthworm powder obtained from vermicomposting unit rather than garden soil. ANOVA analysis (Single factor) at $p = 0.05$, proved that differences between the different concentrations of earthworm powder treatment containing colonies of *C. albicans* was significant, indicating the effect of earthworm powder by inhibiting the growth of the fungus *C. albicans*.

Key words: Earthworm, bacteria, fungi, antimicrobial, antifungal, Guyana

INTRODUCTION

There are approximately 4500 species of worms in the world. Of those about 2500 are earthworm species. In the classification system, earthworms are the larger members of the Oligochaeta in the phylum Annelida. They are placed in the order Opisthopora, on the basis of the male pores opening to the outside of the body posterior to the female pores, even though the male segments are anterior to the female (Ismail, 1997; Edwards and Bohlen, 1996). Earthworms are found virtually worldwide and live in almost any type of soil that contains the right amounts of moisture and organic particles. Earthworms are of various sizes and colors. The color ranges from brownish-black tinge to purple with the exception of some being green. However the most common

color is reddish-brown, which result from the pigment hemoglobin in the blood. The dorsal side of the worm is darker while the ventral side in paler (Mihara *et al*., 1996).

Earthworm has been known for many centuries as a therapeutic drug source for various diseases in China and other parts of the Far East (Ismail, 2005). However, practical pharmacological studies have not been performed except on lumbrofebrin as an antifebrile (Mihara *et al*., 1996). In 1983, it was reported that very strong and novel fibrinolytic enzymes could be extracted from the earthworm, *Lumbricus rubellus*. These enzymes were fractionated and purified as six novel fibrinolytic enzymes and named collectively as Lumbrokinase (Mihara *et al*., 1996; Tang *et al*., 2000; Li *et al*., 2008). It was also found that earthworm powder contains two kinds of inhibitory substances for the platelet aggregation induced by collagen and ADP. One of these inhibitors of platelet aggregation was identified with adenosine. However, the other was a novel substance of MW 260. The structure of this substance was decided on the basis of NMR, mass spectra and infrared spectra. This novel substance also displays a relaxation effect for the canine saphenous vein induced by prostaglandin F *in vitro* and an inhibitory effect on the Active Partial Thromboplastin Time (APTT) (Mihara *et al*., 1996). Earthworm powder can be given orally, thus for this reason, earthworm powder 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). It can be concluded therefore that earthworm powder represents a very promising agent for the treatment of thrombosis (Mihara *et al*., 1996). Its tonic properties also make it beneficial support for the liver and other organ systems. In China, Korea, Vietnam and most of Southeast Asia, *Lumbricus* has been used for their therapeutic benefits for thousands of years and referred to as Earth Dragons. In Korea, these earthworms are believed to promote general health and prevent a wide variety of diseases (El-Kamali, 2000). Furthermore, the earthworm is a primary ingredient in the traditional Vietnamese remedy known as, Miracle Medicine that can Save lives in 60 min. Several Vietnamese-based studies have demonstrated the safety and effectiveness of the earthworm for supporting immunity and cardiovascular health. Because it originates from the soil, the earthworm has dense nutritional content as well as anti-oxidant properties (Mihara *et al*., 1996).

In Guyana, there have been experiments carried out isolating enzymes from the earthworm powder and converting it into dietary supplement, such as Lumbrokinase (Gao and Qin, 1999). Glycolipoprotein (G-90), a mixture obtains from tissue homogenate of *Eisenia foetida* exhibits anti-bacterial properties (Popovic *et al*., 2005). Present study focused on anti-bacterial properties along with the anti-fungal properties in the powder of *Eisenia foetida* which may have applications indirectly implicated in treatment of diseases related to different microbes and fungi.

MATERIALS AND METHODS

The present work was carried out during the year 2006-2007 at University of Guyana, Georgetown. Approximately 2500 of the cultured earthworm, *Eisenia fetida*, were collected. The earthworms were soaked in distilled water for 5 h to allow the soil that was in its tract to be excreted. They were then washed thoroughly with distilled water and place in a Petri dish and place in an incubator for 24 h at a temperature of 55°C. After the 24 h period, the earthworms were removed and pounded to make it into a powder. The powder was stored in a refrigerator at normal temperature (Yegnanarayan *et al*., 1987).

Sulphuric acid and nitric acid digestion method (Homer, 2003) was used for the estimation of various trace elements in the earthworm powder. The trace elements tested for, included the following:

- Magnesium (Mg)
- Calcium (Ca)
- Iron (Fe)
- Manganese (Mn)
- Zinc (Zn)
- Copper (Cu)

The earthworm powder was also subjected to analysis of nitrogen and potassium using standard procedures (Homer, 2003). The analysis of the samples was done at Central Laboratory, Research Center, Agriculture Department, LBI Compound, GuySuCo.

Antimicrobial disc diffusion suspecting tests were carried out. The technique involves using disk diffusion susceptibility testing where disks from both the pure and diluted honey as well as the antibiotic disk erythromycin (control) were impregnated onto the surface of the Mueller Hinton agar. At areas where the concentration of both the diluted and undiluted honey were sufficient to prevent bacterial growth, a distinct margin known as the inhibition zone (Bauer *et al*., 1959) can be seen. Similarly, if the organism tested has no resistance to the antibiotic disk impregnated on agar surface then as the antibiotic diffused from the disk an inhibition zone is formed around the disk. Bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) as well as the fungus *Candida albicans* were administered the following treatments: Control, earthworm powder in water (1:1), earthworm powder in water (1:2) and earthworm powder in acetone (1:2).

All the petri dishes (with replications) were inoculated with respective bacterium and fungus which were isolated from pure cultures. The disks soaked with various concentrations [Control, earthworm powder in water (1:1), earthworm powder in water (1:2) and earthworm powder in acetone (1:2)] were placed on the surface of agar. An antibiotic control disk, erythromycin was also inserted onto the surface of agar. The inoculated plates were incubated for 24 h at 37°C. Each plate was then observed accordingly for any inhibition zones. Inhibition zones present were measured in diameters. The growth of colonies was also estimated for each plate. One of each petri-dish with growing microorganism was left untreated and was used as a control. The treatment was done in triplicate. Before each treatment, the colonies of all of the tested organisms were counted using population estimation charts.

RESULTS

In dried earthworm (*Eisenia fetida*) powder iron is found in the greatest concentration, while magnesium is in the lowest concentration (Table 1). The concentration from highest to lowest is

Table 1: The concentration of various elements present in dried earthworm powder

Elements	Mean±SD
Nitrogen (%)	0.9165±0.0912
Phosphorus (%)	0.4855±0.0290
Potassium (%)	0.4800±0.0071
Iron (ppm)	282.7195±72.6121
Magnesium (ppm)	0.0465±7.0711
Manganese (ppm)	15.3045±0.6201
Zinc (ppm)	95.4470±1.1738
Calcium (ppm)	0.1335±0.0021
Cupper (ppm)	8.1050±1.1639

iron, manganese, zinc, cupper, calcium, potassium, phosphorus and magnesium, respectively. The concentration of the elements analyzed in the earthworm powder differs from that seen in previous experiments (Mihara *et al*., 1996).

Antimicrobial disc diffusion suspecting tests conducted showed that the plates that were inoculated with disks soaked in acetone and dried earthworm powder showed less number of colonies as compared to water at different concentration (Table 2). This small number of colonies is as a result bactericidal effect (cause the death of the organisms). The number of colonies in the plates inoculated with disks soaked in water and earthworm powder was similar to the number seen in the control which is due to bacteriostatic effect (cause temporary inhibition of growth). Dried earthworm (*Eisenia fetida*) powder is only effective against fungus, which was determined by the presence of an inhibition zone (Table 3). The less concentrated the medium in which the disks were soaked, the greater the size of the inhibition zone. Disks soaked in water also had a larger inhibition zone than disks soaked in acetone, because of bactericidal effect while in water the effect was bacteriostatic (Table 4). The ANOVA Single Factor analysis at $p = 0.05$, showed that the F value (27.04) is greater than the F-critical value (5.14), therefore there is significant differences between the different concentration of earthworm powder treatment containing colonies of *C. albicans* which indicate that the earthworm powder is inhibiting the growth of the fungus *C. albicans* (Table 5). However, the dried earthworm powder that was tested had no anti-bacterial properties which may be attributed to difference in composition of elements in earthworm powder obtained from vermicomposting unit. Anova analysis (Single factor) at $p = 0.05$, the $F = 27.04$ is greater than F-crit. = 5.14. Therefore, there is significant differences between the different concentrations of earthworm powder treatment containing colonies of *C. albicans* indicating the effect of earthworm powder by inhibiting the growth of the fungus *C. albicans*. The presence of fungal proteases which act in conjunction with other proteolytic enzyme in the earthworm powder, against the fungus *Candida albicans*.

Table 2: The number of colonies in each plate for the various treatments used

Organisms	Control	Water (1:1)	Water (1:2)	Acetone (1:2)
Staphylococcus aureus	10^6	10^7	10^6	10^3
Escherichia coli	10^6	10^5	10^4	10^3
Pseudomonas aeruginosa	10^5	10^7	10^5	10^4
Candida albicans	10^7	10^6	10^5	10^7

Table 3: Presence or absence of inhibition zone in the plates studied

Organisms	Water (1:1)	Water (1:2)	Acetone (1:2)
Staphylococcus aureus	-	-	-
Escherichia coli	-	-	-
Pseudomonas aeruginosa	-	-	-
Candida albicans	+	+	+

+: Presence of inhibition zone, -: No inhibition zone

Table 4: The length (mm) of inhibition zone seen in the *Candida albicans* inoculated plates

Water (1:1)	Water (1:2)	Acetone (1:2)
43	12	13
30	17	9
33	11	10

Table 5: The statistical analysis of the zone of inhibition seen in the fungus *Candida albicans*

Groups	Count	Sum	Average	Variance
Anova: Single factor				
Column 1	3	106	35.33333	46.33333
Column 2	3	40	13.33333	10.33333
Column 3	3	32	10.66667	4.333333

Source of variation	SS	df	MS	F-value	p-value	F-crit.
ANOVA						
Between groups	1099.556	2	549.7778	27.03825	0.000996	5.143253
Within groups	122	6	20.33333			
Total	1221.556	8				

DISCUSSION

Sherman-Huntoon reported that the magnesium content of vermicompost is relatively low (Hashemimajd *et al*., 1998). This is supported by the analysis carried out on the dried earthworm powder where magnesium was in the lowest concentration. Hisashi Mihara and his collaborators objective was to produce dried earthworm powder by which a pharmaceutically acceptable and highly safe dried earthworm powder exhibiting excellent antihyperlipemic, antidiabetic or hypoglycemic, antihypertensive and/or antihypotensive activities without producing any side effects. The dried garden earthworm powder (*Eisena fetida*) that was produced had approximately 11% soluble nitrogen. In their analysis the element with highest to lowest concentration present in the dried powder was zinc, cupper, manganese, potassium, phosphorus, calcium, magnesium and iron, respectively (Mihara *et al*., 1996). Dickerson (1994) reported that the elements present in worms obtained from vermicomposting unit differ from worms obtain from garden soil. The concentration from highest to lowest is iron, manganese, zinc, cupper, calcium, potassium, phosphorus and magnesium, respectively. The concentration of the elements analyzed in the earthworm powder differs from that seen in previous experiments (Mihara *et al*., 1996).

Mitchell pointed out that 18% of total N from composting material entered earthworm bodies (Hashemimajd *et al*., 1998). This can also be supported by the analysis carried out, where the powder showed relative amount of nitrogen (0.965 mg L^{-1}) present. Especially in the Oriental countries, earthworms (also called dilong) have been used as a drug from remote antiquity. It has been reported that earthworms have a variety of pharmacological activities, i.e., they show gastro protective effect (Prakash and Gunasekaran, 2010), they are effective in reducing the size of vesical calculi and eliminating them from the body, in the treatment of jaundice and as a parturifacient, restrative, hair grower, tonic and an antipyretic. On the other hand, they have also reported some toxic actions of earthworms. That is, earthworm poison injures the nervous systems and causes hemolysis (or the destruction of red blood cells) (Clarke, 1997). Earthworm tincture, that is an ethyl alcohol extract of the earthworm, has a hypotensive effect (Yakugo, 1997). The dried earthworm powder when administered orally to rats and human beings, has an antihyperlipemic effect, a blood sugar lowering effect and a blood pressure regulating effect (i.e., an antihypertensive and/or antihypotensive effect), but the reason for this has not been fully understood. Nevertheless, these effects are believed to be due to the action of the proteolytic enzymes (proteins) contained in the dried earthworm powder, precursors (proteins) of these enzymes, other proteins, lipids or unknown compounds, or a combination thereof (Mihara *et al*., 1996).

Japanese scholars like Mihara *et al*. (1996) succeeded in extracting a fibrin-dissolving enzyme from *Lumbricus rubellus* and found that this enzyme consists of a few proteolytic

sub-enzymes, which are collectively named lumbrokinase. G-90 is a glycolipoprotein mixture obtained from the tissue homogenate of earthworm *Eisenia foetida* (Annelida Concentrations of 10 and 10 μg mL^{-1} of G-90 exhibited an inhibitory effect on *in vitro* growth of non-pathogenic and facultative-pathogenic microbes (Popovic *et al*., 2005; Matausijc-Pisl *et al*., 2010).

The most prevalent trace element present in the dried earthworm powder is iron since its composition is 282.7195 mg L^{-1} as compare to magnesium, the least concentrated element in the powder with 0.0465 mg L^{-1}. The dried earthworm powder obtained from vermicomposting unit had appropriate composition of elements as reported in earlier reports. The presence of fungal proteases which act in conjunction with other proteolytic enzyme in the earthworm powder, against the fungus *Candida albicans* (Popovic *et al*., 2005). It was determined that *Eisenia foetida* have anti-fungal properties. Earthworm powder tested with water is more effective than when tested with acetone.

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