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Effect of Earthworm (*Perionyx excavatus*) Powder on Selected Bacteria and Fungi

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Authors' contributions

This work was carried out in collaboration between all authors. Author GPP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AA, SJ and DS managed the analyses of the study. Author GPP carried out the research work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Investigations were carried out at University of Guyana Turkeyen Campus during the year 2014-15 and focused on the chemical composition of earthworm (*Perionyx excavatus*) powder and its effect on microbes.
Study Design and Methodology: Dried earthworm powder was prepared from adult *Perionyx excavatus* and tested against bacteria (*Pseudomonas aeruginosa* ATCC27583, *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC25923) as well as fungi (*Candida albicans* ATCC24055*; Aspergillus flavus* and *Aspergillus niger*). Petri plates inoculated with known amount of bacterial and fungal colonies on Mueller Hinton agar media were treated with disks impregnated with different concentrations of aqueous solvent extract and ethyl acetate solvent extract of earthworm powder by following the disk diffusion suspecting tests technique. Earthworm powder was subjected to analysis of Magnesium (Mg), Calcium (Ca), Iron (Fe), Manganese (Mn), Zinc (Zn), Nitrogen (N) and Copper (Cu) using standard procedures.
Results: Ethyl acetate extract of earthworm powder at concentration 1:5 was more effective

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against Gram negative aerobic bacteria *Pseudomonas aeruginosa* ATCC27583 while aqueous extract at concentration 1:5 was more effective on Gram negative anaerobic bacteria *Escherichia coli* ATCC25922. Aqueous extract of earthworm powder proved to be more effective on bacteria *Staphylococcus aureus* ATCC25923 in comparison to ethyl acetate extract of earthworm powder. Aqueous extract of earthworm powder had greater antifungal activity on *Candida albicans* ATCC24055 and *Aspergillus flavus,* whereas ethyl acetate extract at higher concentration have shown higher antifungal activity on *Aspergillus niger.*

Conclusion: This study conclusively proved that the earthworm powder prepared from *Perionyx excavatus* has antifungal and antibacterial properties.

Keywords: Perionyx excavates; bacteria; fungi; erythromycin; nystatin.

1. INTRODUCTION

Earthworms were known since 1340 AD and are recognized mostly in Chinese herbology and Asian bodywork therapy to diagnose and treat illness, prevent disease and improve well-being [1]. In 1993, it was reported that fibrinolytic enzymes could be extracted from earthworm, *Lumbricus rubellus*. These enzymes were fractioned and purified as six novel fibrinolytic enzymes collectively named as Lumbrokinase [2]. In the late 1990's the therapeutic and preventive effects of fibrinolytic enzymes on thrombosis-related disease have been clinically confirmed [3]. These enzymes have inhibitory effects on platelet aggregation, anticoagulation effect and relaxation effect for the vascular system. As a result it is used as a thrombotic therapy and represents a promising agent for the treatment of thrombosis itself. It can be orally administered [1]. It was found that earthworm powder contains two kinds of inhibitory substances for the platelet aggregation induced by collagen and adenosine diphosphate (ADP). One of the inhibitors of platelet aggregation was identified with adenosine while the other was a novel substance of MW 260 [2]. The novel substance of MW 260 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) [4].

Earthworms produce several types of leukocytes and synthesize and secrete a range of immune-protective molecules [3]. Their coelomic fluid contains biologically active molecules and leukocytes that participate in the ingestion and killing of bacterial cells in *vitro* [5]. This was proven in *Eisenia spp.* earthworms whose coelomic fluid possesses antibacterial and chemical properties that stop bacteria from reproducing (bacteriostatic) [6]. Earthworms have natural immunity as well as functions associated with the adaptive immunity (allogenic tissue rejection) [3].

Further, antipyretic and anti-oxidative activities have been detected in the *Lumbricus* spp. and *Perichaeta* spp. [3]. High protein, nitrogen and fat content were detected in *Perionyx excavatuss* earthworms all of which work together to regulate the antimicrobial activities. *Lumbricus rubellus* earthworms when tested *in vitro* against a broad spectrum of microorganisms without hemolytic activity showed antimicrobial peptide in the form of lumbricin I. Lumbricin 1 is considered as a proline-rich antimicrobial peptide containing 62 amino acids including 15% proline. Antibacterial peptides were discovered in *Pheretima tschiliensis* and *Eisenia foetida* [3].

In Iran, dried earthworms were prescribed to treat jaundice. Earthworm tonic properties make it beneficial support for the liver and other organ systems. The bacteriolytic substances in earthworms include fetidins, lysenins, lumbricin, eiseniapore, coelomic cytolytic factor (CCF-1) and erythrocytolytic proteins [1]. In China, Korea, Vietnam and most of South East Asia, *Lumbricus* has been used for their therapeutic benefits and recognized in oriental medicine as anti-inflammatory, analgesic and antipyretic agent as well as anticancer agent. In these nations earthworms are referred to as "Earth Dragons" [1].

In Korea, earthworms are believed to promote general health and prevent a wide variety of diseases. In Southeast Asia earthworms are combined with herbs to tonify the sympathetic and parasympathetic functions of the central nervous system and at same the time support digestive functioning in the stomach and the gastrointestinal tract [4].

Further in Vietnam, it is traditionally referred to as a primary ingredient in Miracle Medicine which is capable enough for saving life in 60 minutes. There are numerous Vietnamese-based studies that have shown the effectiveness of earthworm for supporting immunity and cardiovascular

health owing to the fact that it has dense nutritional content and anti-oxidant properties [2].

In ancient Burma and Laos, smallpox victims were doused with water which had earthworms soaked in it. This is a remedy to treat various diseases. These worms were boiled in water together with salt and onions and the broth is fed to women with postpartum weakness or difficulty nursing [1].

Apart from medicinal values, earthworms have been used as food for humans especially in the diet of Yekuana people from Venezuela. They have high nutritive value and contain about 60 to 70% protein. Besides the human diet, earthworms have been used for feeding fish [3]. Further studies on the importance of earthworms in areas such as biotechnology, search of vermi-composting process, eco-toxicology, ecology, taxonomy and morphology, soil physics and soil fertility were conducted during the past year [7,8].

Moreover, as euedaphic living being they spend their entire life cycle within the soil. By burrowing, casting, feeding and propagating, they directly or indirectly influence the physical and chemical properties of the soil in many ways and establish the basis for other organisms such as micro-organisms, other groups of soil animals and plants [1]. Besides this they are valuable and low cost source of many bioactive molecules, which could find place in human and veterinary medicine. Thus, it is obvious that earthworms have a whole variety of application, from environmental protection, medical use and nutritional production.

Since earthworms coexist with micro-organisms, it is of great interest to find out the efficacy of earthworm powder on bacteria and fungi and the chemical composition of earthworm powder that contribute towards such effect. In this investigation, antimicrobial disk diffusion tests were carried out against bacteria (*Pseudomonas aeruginosa* ATCC27583, *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC25923) as well as fungi (*Candida albicans* ATCC24055*; Aspergillus flavus* and *Aspergillus niger*).

2. MATERIALS AND METHODS

Three hundred (300) cultured earthworms were used. The earthworms were placed into distilled water for nine (9) hours to allow the soil that is in its tracks to be excreted. After nine hours they were washed thoroughly with distilled water and placed into a Petri dish which was then incubated for 24 hours at a temperature of 55℃. After 24 hours, the earthworms were removed and pounded into powder. The powder was stored in a refrigerator at normal temperature [9]. Sulphuric acid and nitric acid digestion method was used for the estimation of various trace elements in the earthworm powder. The trace elements tested are Magnesium (Mg), Calcium (Ca), Iron (Fe), Manganese (Mn), Zinc (Zn), Nitrogen (N) and Copper (Cu) using standard procedure (Homer, 2003).

Sulphuric acid/nitric acid digestion was carried out for testing the presence of various trace elements (Mg, Ca, Fe, Mn, Zn and Cu) in earthworm powder. 0.5 g of the dried earthworm powder was carefully weighed and placed into a 250 mL conical flask.15 mL of digestion reagent was added to the flask. A blank sample and a reference sample were digested in parallel with the test sample. The solution was digested on a hot plate in a fume hood until the solution become clear. It was digested to a solution volume of approximately 5 mL. The sample was allowed to cool for approximately 15-20 minutes. The digest was quantitatively transferred to a 100 mL volumetric flask. The digest was diluted to the 100 mL mark with distilled water. This was then shaken vigorously and left to stand for approximately one hour. With the use of an Atomic Absorption Spectrophotometer (AAS) the concentration of the trace elements were evaluated.

Nitrogen, phosphorus and potassium were determined by taking 0.1g of dried earthworm powder weighed and placed into a clean boiling tube containing a small glass bead. The following was added to the boiling tube (1.0 g Sodium sulphate, 7 standard drops of Copper sulphate solution; and 3mL Sulphuric acid).

The boiling tube was covered with a glass bulb and the content was mixed by gently shaking the tube. The tube was placed on a thermoelectric heater and was boiled for 2 hours at 390℃ until a clear greenish solution was obtained. The solution was left to cool to room temperature. The solution was then washed with distilled water and filtered through a bed of glass wool into a 100 mL volumetric flask. The flask was covered with a stopper and was carefully shaken to prepare a homogenous mixture. It was then left to settle for one hour. Analysis for Nitrogen, Phosphorus and Potassium on aliquot of this sample was done. Colorimetric comparison was

made at 420 nm wavelength spectrophotometrically on the Helger photometer before flocculation occurred.

2.1 Preparation of Aqueous Extract of Earthworm Powder

16 grams of earthworm powder was accurately weighed and placed into a beaker containing 16mL of purified water (1:1). The solution was then boiled and filtered through standard Whatman No.1 filter paper. The filtrate obtained was subsequently evaporated on a hot plate until it reaches the concentrated quantity [1].

2.2 Preparation of Ethyl Acetate Extract of Earthworm Powder

16 grams of earthworm powder was accurately weighed and placed into a beaker containing 16 mL ethyl acetate (1:1). The mixture was then filtered and the filtrates obtained were condensed in water- bath at 35℃. This process was repeated until sufficient quantity occurs [1].

2.3 Culture Media, Inoculum and Plating

Mueller Hinton II Agar was used as the base for pure cultures and Antimicrobial Disk Diffusion Susceptibility Testing. Two loopful of pure cultured organisms were added to test tubes containing 5mL of broth media and was mixed thoroughly so as to evenly distribute the colonies (Stokes, 1980). The bacterial cultures were incubated at 37℃ for 18 hours while the fungal cultures were incubated at 37℃ for 48 hours [1]. The overnight cultures were diluted using serial dilutions tube method to obtain suspensions of 10^5CFU/mL. Viable colony forming units were enumerated by plating the serially diluted cells [10]. The spread plated technique was used to evenly distribute 0.1mL the serially diluted cells on the culture media [11].

2.4 Antimicrobial Activity

Mueller-Hinton agar media was inoculated with suspensions of 10^5CFU/mL which was evenly distributed throughout the media by the spread plate technique [11]. 200 µl of different concentrations of earthworm extract were placed on to Whatman No.1, 6 mm diameter filter paper discs [1]. The concentration of earthworm extract used include: 5mg of earthworm powder extract to 5mL of sterile water (1:1), 15 mg of earthworm powder extract to 5mL of sterile water (1:3), 25 mg of earthworm powder extract to 5mL of sterile water (1:5), 5 mg of earthworm powder extract to 5 mL of ethyl acetate (1:1), 15mg of earthworm powder extract to 5mL of ethyl acetate (1:3) and 25 mg of earthworm powder extract to 5 mL of s ethyl acetate (1:5). The filter paper discs were allowed to completely dry and was later used to treat the inoculated agar. Standard Erythromycin and Neomycin was used as reference for bacteria and fungi respectively. Water and ethyl acetate was used as controls. The antibacterial and antifungal activity was assessed by measuring the diameter of inhibition zone in millimeters. All tests were performed in triplicate. Before and after treatment the colony forming units were counted [2].

3. RESULTS

In dried earthworm (*Perionyx excavatus*) powder nitrogen is found in the greatest concentration. The percentage of elements ranging from second highest to lowest in dried *Perionyx excavatus* powder were Potassium, Phosphorus, Calcium, Iron, Magnesium, Zinc, Manganese and Copper (Table 1). This is in contrary to previous studies carried out on earthworm powder prepared from *Eisena foetida* and *Lampito mauritii* powder [2,1]. Antibacterial and antifungal activities were detected from the formation of inhibition zone after the inoculated plates were treated with antimicrobial disks soaked with different concentrations of earthworm extract.

Results have shown that ethyl acetate extract of earthworm powder at concentration 1:5 is more effective against Gram negative aerobic bacteria *Pseudomonas aeruginosa* ATCC27583 while aqueous extract at concentration 1:5 is more effective on Gram negative anaerobic bacteria *Escherichia coli* ATCC25922. Aqueous extract of earthworm powder proved to be more effective on bacteria *Staphylococcus aureus* ATCC25923 in comparison to ethyl acetate extract of earthworm powder. Additionally, aqueous extract of earthworm powder has revealed higher antifungal activity on *Candida albicans* ATCC24055 and *Aspergillus flavus.* In contrast ethyl acetate extract at higher concentration have shown higher antifungal activity on *Aspergillus niger* and proved to possess more antifungal activity compared to that of Nystatin (Tables 2, 4, 6, 8 and 10 and Figs. 1-32).

The ANOVA single factor analysis at $P \leq 0.05$, showed that the F value is higher than the Fcrit value for all bacteria and fungi (Tables 3, 5, 7

and 9). This indicates that earthworm powder is inhibiting the growth of all bacteria and fungi that was investigated. Therefore earthworm powder has significant effect on all bacteria and fungi in question.

Table 1. Nutrient status of earthworm powder, *Perionyx excavates*

Elements	Percentage (%)
Nitrogen (N)	7.38
Phosphorus (P)	0.73
Potassium (k)	0.93
Calcium (Ca)	0.3055
Copper (Cu)	0.000114
Iron (Fe)	0.1054
Magnesium (Mg)	0.1013
Manganese (Mn)	0.00443
Zinc (Zn)	0.0063

The final colony forming units were calculated based on the width of inhibition zone after the microbial plates were treated with different concentrations of earthworm extract. With reference to the final colony forming unit calculated, ethyl acetate solvent extract of earthworm powder showed strong antibacterial effect against *Escherichia coli* and *Pseudomonas aeruginosa* as compared to that of aqueous solution. However, aqueous extract of earthworm powder showed high antibacterial effect against *Staphylococcus aureus.* It is evident that aqueous extracts of earthworm powder possess antifungal effect against *Aspergillus flavus* while for *Candida albicans* and *Aspergillus niger* no final colony forming unit was estimated due to fact that the colonies were too numerous to count.

4. DISCUSSION

The present investigation was successfully completed with the collection and culture of the earthworm species *Perionyx excavatus*, and then the converted powdered form was subjected to organic chemical analysis. Numerous scientists have reported high levels of nutrients in powdered earthworms [12,13]. The organic chemical composition of earthworm *(Perionyx excavatus)* powder is quantified in Table 1.

This research revealed that nitrogen showed the highest percentage which account for 7.38 % of dry earthworm powder and thus signify the highest nutritional value. This is contrary to previous studies carried out on earthworm powder prepared from *Eisena foetida* and *Lampito mauritii.* According to research conducted by [2] on dried earthworm (*Eisena foetida)* powder, iron showed the highest concentration. The dried garden earthworm powder (*Eisena foetida*) had approximately 11% soluble nitrogen. Additionally, research conducted by [1] found that iron was in the greatest concentration for earthworm (*Lampito mauritii)* powder while nitrogen was the fifth highest. However, the concentration of the elements analyzed in *Perionyx excavatus* powder turn out to be different from that seen in other investigations using different species.

Previous research conducted by [2] revealed that magnesium in dried earthworm (*Eisena foetida)* powder had the lowest concentration. By comparison, Copper in *Perionyx excavates* powder showed the least concentration which account for 0.000114% of the dried weight of the

Table 2. Showing the width (mm) of inhibition zone in plates inoculated with bacteria (aqueous extract)

Bacteria	Diameter (mm) of inhibition zone				
	Water only (control)	Water 1:1	Water 1:3	Water 1:5	Erythromycin (reference)
	0	11	13	14	18
Staphylococcus aureus ATCC25923	0	12	14	16	20
	0	11	13	14	18
	0	0	0	15	20
Escherichia coli ATCC25922	0	0	0	20	20
	0	0	0	20	20
Pseudomonas aeruginosa ATCC27583	0	11	13	15	20
	0	11	13	14	19
	0	11	12	15	19

Table 3. Statistical analysis for bacteria treated with aqueous extract

ANOVA: Single factor						
Summary						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Control	9	0	0	0		
Water 1:1	9	67	7.444444	31.27778		
Water 1:3	9	78	8.666667	42.5		
Water 1:5	9	143	15.88889	5.861111		
Reference (Erythromycin)	9	174	19.33333	0.75		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2067.689	4	516.9222	32.15135	5.19E-12	2.605975
Within Groups	643.1111	40	16.07778			
Total	2710.8	44				

Table 4. Width (mm) of inhibition zone in plates inoculated with bacteria (Ethyl acetate)

Bacteria	**Diameter (mm) of inhibition zone**				
	Ethyl acetate only (control)	**Ethyl acetate 1:1**	**Ethyl acetate 1:3**	**Ethyl acetate 1:5**	**Erythromycin (reference)**
	0	8	8	9	10
Staphylococcus aureus	0	8	9	10	12
ATCC25923	0	0	0	10	12
	0	10	13	14	30
Escherichia coli ATCC25922	0	11	13	14	30
	0	10	13	14	30
Pseudomonas aeruginosa	10	12	12	15	15
ATCC27583	10	12	15	16	20
	9	11	12	12	19

Table 5. Statistical analysis for bacteria treated with ethyl acetate extract

ANOVA: Single factor						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Control	9	29	3.222222	23.44444		
Ethyl acetate 1:1	9	82	9.111111	13.86111		
Ethyl acetate 1:3	9	95	10.55556	20.27778		
Ethyl acetate 1:5	9	114	12.66667	6.25		
Reference (Erythromycin)	9	178	19.77778	69.19444		
ANOVA						
Source of variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1296.578	4	324.1444	12.18334	1.44E-06	2.605975
Within Groups	1064.222	40	26.60556			
Total	2360.8	44				

worm. Magnesium in *Perionyx excavatus* account for 0.1013% of the total dried weight while Potassium (k) being 0.93%; phosphorous being 0.73%; Calcium (Ca) being 0.3055%; Iron (Fe) being 0.1054%; Zinc (Zn) being 0.0063% and Manganese (Mn) being 0.00443%. The research conducted on macro-minerals of four species of earthworms, the occurrence of potassium (0.93%) and phosphorus (0.73%) in present study further suggests that *Perionyx excavatus* is likely to have a high concentration of crude protein. The reason for

this is that phosphorous play an important role in energy metabolism which has an effect on carbohydrates, lipids and protein [1]. Although iron occurs as the fifth highest bio-available nutrient in earthworm *Perionyx excavatus* powder, it can be useful food source for iron deficiency anemia. The presence of 0.3055% calcium can be important for pregnant and lactating mothers and essential for blood clotting and muscle contraction [1]. Potassium is important for DNA, protein synthesis and cell volume regulation [14].

Earthworms as protein source had been established by several authors [15-18]. [19] found that *Perionyx excavatus* is a good source of protein. Similarly, [15] found that *Dendrodrilus subrubicundus* contains 65% crude protein.

Table 6. The width (mm) of inhibition zone in plates inoculated with fungi (aqueous extract)

Fungi	Diameter (mm) of inhibition zone				
	Water only (control)	Water 1:1	Water 1:3	Water 1:5	Erythromycin (reference)
	0	13	13	14	14
Candida albicans ATCC24055	0	15	20	22	20
	0	13	14	15	10
	0	11	16	18	10
Aspergillus flavus	0	13	16	17	10
	0	11	16	17	9
Aspergillus niger	0	11	13	15	9
	0	12	14	15	10
	0	11	14	14	9

Table 7. Statistical analysis for fungi treated with aqueous extract

ANOVA: Single factor						
Summary						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Control	9	0	0	0		
Water 1:1	9	110	12.22222	1.944444		
Water 1:3	9	136	15.11111	4.861111		
Water 1:5	9	147	16.33333	6.5		
Reference (Nystatin)	9	101	11.22222	13.19444		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1510.978	4	377.7444	71.27254	1.17E-17	2.605975
Within Groups	212	40	5.3			
Total	1722.978	44				

Table 8. The width (mm) of inhibition zone in plates inoculated with fungi (ethyl acetate)

Fungi	Diameter (mm) of inhibition zone				
	Ethyl acetate only (control)	Ethyl acetate 1:1	Ethyl acetate 1:3	Ethyl acetate 1:5	Erythromycin (reference)
	0	8	8	9	10
Staphylococcus aureus	0	8	9	10	12
ATCC25923	0	0	0	10	12
	0	10	13	14	30
Escherichia coli ATCC25922	0	11	13	14	30
	0	10	13	14	30
Pseudomonas aeruginosa	10	12	12	15	15
ATCC27583	10	12	15	16	20
	9	11	12	12	19

Table 9. Statistical analysis for fungi treated with ethyl acetate extract

ANOVA: Single factor						
Summary						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Column 1	9	29	3.2222222	23.44444		
Column 2	9	82	9.1111111	13.86111		
Column 3	9	95	10.555556	20.27778		
Column 4	9	114	12.666667	6.25		
Column 5	9	178	19.777778	69.19444		
ANOVA						
Source of variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1296.578	4	324.14444	12.18334	1.439E-06	2.605974949
Within Groups	1064.222	40	26.605556			
Total	2360.8	44				

Table 10. Final colony forming unit (CFU) per mL after treatment

Microorganisms used	**Initial CFU/mL**	**Trial 1 (Final CFU/mL)**		**Trial 2 (Final CFU/mL)**		**Trial 3 (Final CFU/mL)**	
		Solvent water	**Solvent ethyl acetate**	**Solvent water**	**Solvent ethyl acetate**	**Solvent water**	**Solvent ethyl acetate**
Escherichia coli	10^5	65,035	33,334	60,000	31,973	60,000	33,334
Staphylococcus aureus	10^5	44,136	64,913	37,889	60,938	44,135	77,974
Pseudomonas aeruginosa	10^5	40,829	35,898	42,858	26,471	42,858	36,709
Candida albicans	10^5	TNC	TNC	TNC	TNC	TNC	TNC
Aspergillus flavus	10^5	44,445	90,000	43,821	90,000	46, 809	90,000
Aspergillus niger	105	TNC	TNC	TNC	TNC	TNC	TNC

**TNC –Too numerous to count*

Studies have shown that earthworm meal of *Lumbricus terrestris* contain 32.60% protein [20], earthworm meal of *Perionyx excavatus* contain 57.2% crude protein [21] and earthworm powder of *Eudrilus eugeniae* contain 5.21 mg/g of protein [22]. Additionally, [22] believed that on an average, dried earthworm powder contain 60-70% of crude protein. The high reproductive rate and biomass production of this tropical earthworm species makes it ideally suited for fish meal production [16].

Another essential point about earthworms is that they contain considerable amount of other minerals that are nutritionally important. As such, they can be used as nutritional applications as animal feed and as an ingredient in food products for humans. They have already been used in experimental diets for rainbow trout and as chicken feed [23,24].

Moreover, earthworms have been used in medicine for various remedies [25]. In the present investigation, water and ethyl acetate solvent extracts of earthworm, *Perionyx excavatus* were prepared and antifungal and antibacterial activity of these extracts were determined by disk diffusion method.

Earthworm powder was tested against three bacteria, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* and three fungi, *Candida albicans, Aspergillus flavus* and *Aspergillus niger*. Based on the results, it is evident that earthworm powder has antifungal and antibacterial effect. Larger width of inhibition zone was noted in plates inoculated with *Candida albicans* after treated with aqueous solvent of earthworm extract. It was noted that ethyl acetate solvent extract of earthworm powder has no antifungal effect on *Appergillus flavus*. When the final colony forming unit per milliliter was calculated, ethyl acetate solvent extract have stronger antibacterial effect on *Pseudomonas aeruginosa* noted by a drastic decrease in the colony forming units from

100,000 CFU/mL to 26,471 CFU/mL. Final colony forming units per milliliter was not calculated for fungi *Candida albicans* and *Aspergillus niger* because the colony forming units were too numerous to count. However, based on the width of inhibition zone measured in millimeters, earthworm powder possesses maximum antifungal activity against *Candida albicans* when treated with aqueous extract. The measurement of inhibition zones revealed that earthworm powder of *Perionyx excavatus* was least effective against *Aspergillus flavus.* Overall the diameter of zone of inhibition observed was 22 mm for *Candida albicans* which is followed by 0mm for *Aspergillus flavus*. This study was supported by previous research conducted by [2] who reported that dried earthworm powder showed antifungal activity against *Candida albicans.*

Erythromycin which was used as positive control for bacteria possesses similar antibacterial activity with earthworm powder at ratio 1:5 for some of the trials. Nystatin was used as reference for fungi and possesses similar antifungal activity when compared with earthworm powder tested against *Candida albicans* in trials 2 at concentration 1:5, using water as a solvent. Most importantly, aqueous extract of earthworm powder at concentrations 1:5 showed higher antifungal activities on fungi *Aspergillus flavus* and *Aspergillus niger* when compared to that of Nystatin*.* This study is supported by [1] who reported that extract of earthworm is an antifungal agent against *Candida albicans.* On the other hand, this study is contrary to research conducted by [25] who reported that the petroleum ether extract of earthworm powder was found to possess maximum antifungal activity against *Aspergillus niger* compared to *Candida albicans.*

Based on findings earthworm powders have significant effects on bacteria and fungi. This is because it is comprised of certain lytic compounds [26]. Lytic compounds are assumed to be antibiotic peptides that inhibit the growth of microorganisms through pathways different from conventional antibiotics [27]. According to [28] antimicrobial agents of earthworm's digestive fluid such as flavonoid, phenol and its lytic compounds are formed in the earthworm body but not by the soil microorganisms entering their digestive tract. Further, [29] reported that that the coelomic fluid of *E. eugeniae* has maximum antibacterial activity against *Staphylococcus aureus.* This is contrary to the results obtained in this investigation which prove that ethyl acetate solvent extract of earthworm *Perionyx excavatus* powder has maximum effective against bacteria *Pseudomonas aeruginosa* which showed a drastic decrease in the colony forming units from 100,000 CFU/mL to 26,471 CFU/mL.

Over the past twenty years, bacteria have acquired resistances to many common antibiotics. In fact, many bacterial pathogens found in hospitals have multiple antibiotic resistances. Bacteria have become resistant by circumventing the specific pathways that antibiotics are designed to inhibit [30]. Owing to the fact that earthworm (*Perionyx excavatus)* powder possesses antibacterial and antifungal activities, this study may lead to the formulation of new antimicrobial drugs.

5. CONCLUSION

This study demonstrates that earthworm (*Perionyx excavatus*) powder have anti-bacterial and anti-fungal properties. The output of this experiment can be extensively used in biomedicine to treat microbial infections and diseases as it relates to different bacterial and fungal infections some of which might be naturally resistant to antibiotics. This study provides clues to the role of earthworm powder in treating the pathogenic effects of different microbes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Fig. 1. Aqueous solvent of earthworm extract

Fig. 2. Ethyl acetate solvent of earthworm extract

Figures showing antibacterial activity of earthworm powder on *Escherichia coli* represented by zone of inhibition.

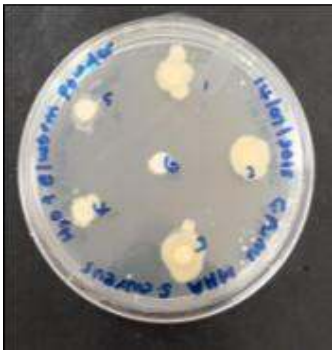

Fig. 3

Fig. 4

Fig. 5

A. Aqueous solvent of earthworm extract

Fig. 6

Fig. 7

Fig. 8

B. Ethyl acetate solvent of earthworm extract

Figures showing antibacterial activity of earthworm powder on *Staphylococcus aureus* represented by zone of inhibition

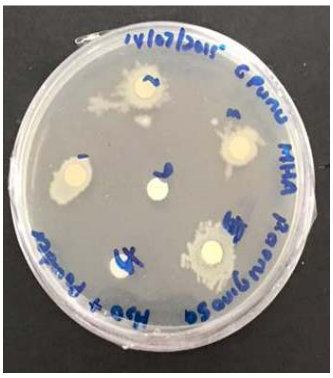

Fig. 9

Fig. 10

Fig. 11

A. Aqueous solvent of earthworm extract

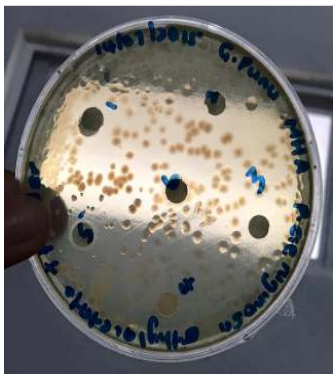

Fig. 12

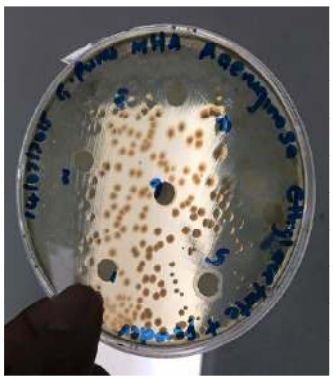

Fig. 13

Fig. 14

B. Ethyl acetate solvent of earthworm extract

Figures showing antibacterial activity of earthworm powder on *Pseudomonas aeruginosa* represented by zone of inhibition

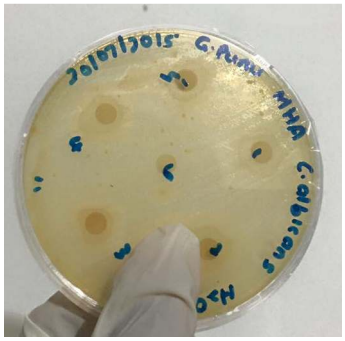

Fig. 15

Fig. 16

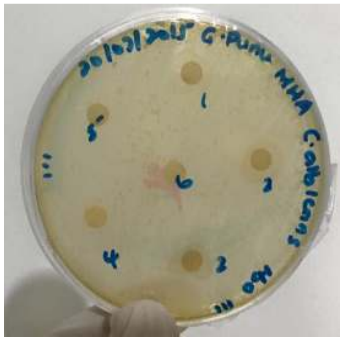

Fig. 17

A. Aqueous solvent of earthworm extract

Fig. 18

Fig. 19

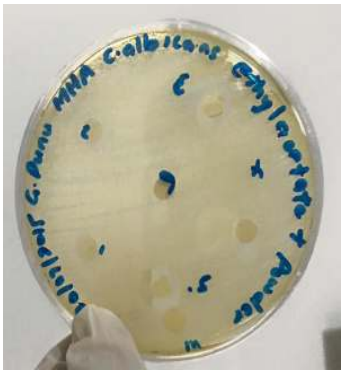

Fig. 20

B. Ethyl acetate solvent of earthworm extracts

Figures showing antifungal activity of earthworm powder on *Candida albicans* represented by zone of inhibition.

Fig. 21

Fig. 22

Fig. 23

A. Aqueous solvent of earthworm extract

Fig. 24

Fig. 25

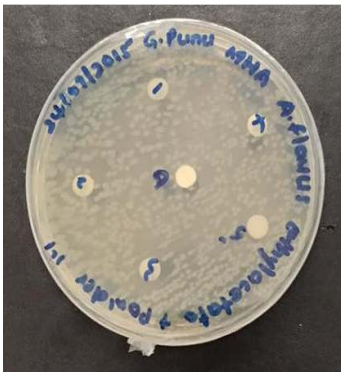

Fig. 26

B. Ethyl acetate solvent of earthworm extract

Figures showing the antifungal activity of earthworm powder on *Aspergillus flavus* represented by zone of inhibition.

Fig. 27

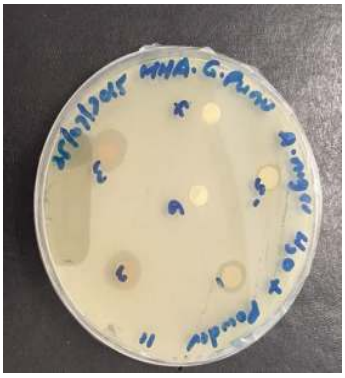

Fig. 28

Fig. 29

A. Aqueous solvent of earthworm extract

Fig. 30

Fig. 31

Fig. 32

B. Ethyl acetate solvent of earthworm extract

Figures showing antifungal activity of earthworm powder on *Aspergillus niger* represented by zone of inhibition.

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