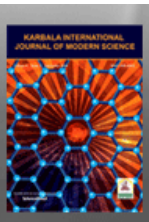

Karbala International Journal of Modern Science

Volume 8 | Issue 3 Article 8

Antimicrobial and Cytotoxic Properties of Extracts from Over-seasoned Worm-casts of the Earthworm Hyperiodrilus Africanus Beddard, 1891

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Recommended Citation

Owa, Stephen Olugbemiga; Otohinoyi, David Adeiza; Edewor-Ikupoyi, Theresa Ibibia; Taiwo, Adeolu Adeola; Okosun, Jenifer Akhere; Akujobi, Yvonne Oluchi; Oyewale, Damilola Grace; Olaolu, Tomilola Debby; Rotimi, Damilare Emmanuel; Osemwegie, Osarenkhoe Omorefosa; and Adeyemi, Oluyomi Stephen (2022) "Antimicrobial and Cytotoxic Properties of Extracts from Over-seasoned Worm-casts of the Earthworm Hyperiodrilus Africanus Beddard, 1891," *Karbala International Journal of Modern Science*: Vol. 8 : Iss. 3 , Article 8.
Available at: https://doi.org/10.33640/2405-609X.3246

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Abstract

• Background: Despite the continuous interest in the search for therapeutic agents, little attention has been given to the medicinal relevance of earthworm casts, with even less interest in over-seasoned worm-casts. Therefore, this study determined the phytochemical, antimicrobial, and cytotoxic properties of over-seasoned worm-casts of the earthworm Hyperiodrilus africanus (Eudrilidae). Methods: The earthworm casts were extracted with n-hexane, ethanol, and water and the crude extracts were evaluated for the presence of chemical constituents and antimicrobial properties. Cytotoxicity was inferred from the antimitotic effects of the extracts on the radicles of germinating seeds of Sorghum bicolor. Results: The chemical constituent determinations revealed the presence of alkaloids, anthraquinones, coumarins, steroids, terpenoids, tannins, cardiac glycosides, and phenols. Screening the extracts for chemical constituents revealed that the ethanolic and aqueous extracts had more chemical constituents than n-hexane extracts. The ethanolic extract showed antibacterial activity against Streptococcus sp. and Staphylococcus aureus; the aqueous extract showed antifungal activity against Aspergillus flavus and Aspergillus niger. Furthermore, both extracts showed antimitotic activity against healthy cells of S. bicolor in a manner similar to that of the reference drug (cyclophosphamide). Conclusion: The study provides evidence, lending credence to the antimicrobial and cytotoxic potential of over-seasoned casts of H. africanus.

Keywords

Anticancer; Medicinal biochemistry; Drug discovery; Phytochemicals

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RESEARCH PAPER

Antimicrobial and Cytotoxic Properties of Extracts from Over-seasoned Worm-casts of the Earthworm *Hyperiodrilus Africanus* Beddard, 1891

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Abstract

Background: **Despite the continuous interest in the search for therapeutic agents, little attention has been given to the medicinal relevance of earthworm casts, with even less interest in over-seasoned worm-casts. Therefore, this study determined the phytochemical, antimicrobial, and cytotoxic properties of over-seasoned worm-casts of the earthworm *Hyperiodrilus africanus* (Eudrilidae).**

Methods: **The earthworm casts were extracted with *n*-hexane, ethanol, and water and the crude extracts were evaluated for the presence of chemical constituents and antimicrobial properties. Cytotoxicity was inferred from the antimitotic effects of the extracts on the radicles of germinating seeds of *Sorghum bicolor*.**

Results: **The chemical constituent determinations revealed the presence of alkaloids, anthraquinones, coumarins, steroids, terpenoids, tannins, cardiac glycosides, and phenols. Screening the extracts for chemical constituents revealed that the ethanolic and aqueous extracts had more chemical constituents than n-hexane extracts. The ethanolic extract showed antibacterial activity against *Streptococcus* sp. and *Staphylococcus aureus*; the aqueous extract showed antifungal activity against *Aspergillus flavus* and *Aspergillus niger*. Furthermore, both extracts showed antimitotic activity against healthy cells of *S. bicolor* in a manner similar to that of the reference drug (cyclophosphamide).**

Conclusion: **The study provides evidence, lending credence to the antimicrobial and cytotoxic potential of over-seasoned casts of *H. africanus*.**

Keywords: Anticancer, Medicinal biochemistry, Drug discovery, *Phytochemicals*

1. Introduction

As a biodiversity component, earthworms accomplish important roles for soil ecosystems and global ecosystems, the widely known being the increasing of soil fertility, the improvement of soil physical−chemical properties, and the waste management (through vermicomposting) [1]. In addition Earthworms play an important role in supporting nutrient cycling as well as creating and stabilizing soil structure and texture [1]. The cultivation effect of earthworms is both a result of their burrowing activity and the consequence of their casting and mucus secretion [2]. These activities affect root growth and water balance as well as the microflora and microfauna, thereby influencing the types of microorganisms present in the soil [1−3].

Received 1 March 2022; revised 16 June 2022; accepted 19 June 2022.
Available online 1 August 2022

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https://doi.org/10.33640/2405-609X.3246

Earthworm casts, also known as worm-casts, are biologically active mounds containing various bacteria (nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and actinomycetes), enzymes, humus, micronutrients, growth hormones (auxins, gibberellins, and cytokinins) and remnants of plant materials and animal manure, which are either secreted, unabsorbed, or undigested by the earthworm [4]. Worm-casts significantly affect plant growth, soil aggregation, and nutrient supply. Activities of β-glucosidase, alkaline phosphatase, dehydrogenase, nitrogenase, protease, amylase, phosphatase, urease, lipase, cellulase, and chitinase have been detected in earthworm casts [4]. Besides, earthworm casts have been reported to have high retention rate for nutrients such as calcium, potassium, iron, sulfur, and potassium. This is due to their high humic acid content, as this provides binding sites for nutrients and moisture, thereby making them frequently referred to as non-pollutant natural fertilizers [4]. Interestingly, worm-casts play a major role in inducing the biological resistance of plants to diseases [5]. For example, chitinase content of worm-casts confers plants with the ability to repel pests since chitinase breaks down chitin in arthropod pests' exoskeletons [5]. Worm-casts are usually produced seasonally by earthworms, especially during the rainy season (typical of West Africa), wherein they are referred to as seasonal or fresh worm-casts. However, casts produced in a particular season may remain in the environment for long periods, and such are referred to as 'over-seasoned casts' [6].

Chemical compounds with antimitotic properties, that affect microtubule dynamics, could serve as anticancer agents. This fact has led to the screening of various chemical compounds for antimitotic properties as a cytotoxic test for the identification of anticancer agents, while the antimicrobial activities could serve as a traditional remedy for various enteric conditions [7]. Despite the continuous interest in the search for therapeutic agents, little attention has been given to the medicinal relevance of earthworm casts, with even less interest in over-seasoned worm-casts. Therefore, in this study, we evaluated over-seasoned casts of *Hyperiodrilus africanus* for medicinal properties by evaluating its cytotoxic and antimicrobial properties.

2. Materials and methods

2.1. Chemicals and reagents

n-hexane, ethanol, peptone, sodium chloride, and nutrient agar were obtained from Sigma–Aldrich (Missouri, USA). All other chemicals and reagents used were of analytical grade (Sigma–Aldrich, Missouri, USA)

2.2. Cast collection

In February 2014, over-seasoned turret casts of the earthworm *H. africanus* (Eudrilidae) were collected from abandoned farmland at Obbo-Ile in Kwara State, Nigeria (8.08333°N, 5.33333°W). Obbo-Ile is located in a tropical savanna with an extreme seasonal variation: the average temperature range is 20–32 °C, the humidity range is 5–90%, the monthly precipitation range is 3–198 mm, and the probability of rainfall range is 1–83%. When the samples were collected (February), the average temperature was 33 °C, the humidity was 40%, the monthly precipitation was 13 mm, and the probability of rainfall was 8%.

2.3. Preparation of extracts

The extraction process was conducted as described elsewhere [8,9]. Briefly, 1 kg of the worm-casts of *H. africanus* was allowed to dry completely before grounding and sieving. The powder sample was extracted in 2 dm^3 of n-hexane, ethanol, or water at room temperature for 48 h. The extracts were concentrated at 1 atm in a rotary evaporator at varying temperatures for *n*-hexane, ethanol, and water, respectively.

2.3.1. Analysis of chemical constituents

Each extract was evaluated for the presence of secondary metabolites by using protocols described elsewhere [10–13] (Table 1).

2.4. Antibacterial assay (agar well diffusion)

As described by Vasanthi et al. [14], antibacterial activity was determined (using the agar diffusion assay method with a 6-mm sterile metal borer) against *Escherichia coli, Staphylococcus aureus, Streptococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Proteus* sp. The bacterial strains were obtained from the Microbiology Laboratory of Landmark University, Omu-Aran, Kwara State, Nigeria. Microorganisms were inoculated on nutrient broth (containing 1 g/L meat extract, 5 g/L peptone, and 5 g/L sodium chloride) 24 h before the test at 37 °C. The bacterial strains were seeded on nutrient agar (Sigma–Aldrich, Missouri, USA). The wells were loaded with each extract at a concentration of 10 mg/mL. Ethanol was used as a positive control. The plates were incubated at 37 °C for 24 h in a bench-top laboratory incubator (DNP 9052, Sanfa, China). All

Table 1. Methods used to identify chemical constituents of earthworm casts.

Type of test	Name of test	References
Alkaloids	Mayer test	Harborne [11]
Saponins	Froth test	Harborne [11]
Anthraquinones	Borntrager test	Trease & Evans [12]
Coumarins	Fluorescence test	Trease & Evans [12]
Sterols & terpenes	Liebermann–Burchard test	Trease & Evans [12]
Steroids	Liebermann–Burchard test	Trease & Evans [12]
Terpenoids	Salkowski test	Harborne [11]
Flavonoids	Ammonium chloride test	Khan et al. [13]
	Potassium hydroxide test	Khan et al. [13]
	Ammonia test	Khan et al. [13]
	Ethyl acetate test	Khan et al. [13]
Tannins	Braymer test	Trease & Evans [11]
Phlobatannins	Hydrochloric acid test	Sofowora [10]
Cardiac glycosides	Keller–Killani test	Khan et al. [13]
Phenols	Ferric chloride test	Khan et al. [13]

the assays were carried out in triplicate. The diameter (mm) of the growth inhibition zone was measured with a standard zone reader scale and the mean diameter was recorded.

2.5. Antifungal assay (agar well diffusion)

The method described by Vasanthi et al. [14] was used to assess antifungal activity against *Aspergillus niger* and *Aspergillus flavus.* The fungi isolates were obtained from the Microbiology Laboratory of Landmark University, Omu-Aran, Kwara State, Nigeria. The microorganisms were inoculated in potato dextrose broth at 25 °C for 24 h before the test. Wells were made using a sterile metal borer (6 mm). The wells were loaded with each extract at a concentration of 10 mg/mL. Ethanol, which is an antifungal agent, was used as the positive control. The plates were incubated at 25 °C for 48 h, and then all the assays were carried out in triplicate. The diameter (mm) of the growth inhibition zone was measured with a standard zone reader scale and the mean diameter was recorded.

2.6. Minimum inhibitory concentration (MIC)

The method described by Wei et al. [15] was used. The MIC values of the worm-cast extract that gave total inhibition against bacterial isolates were determined using a 2-fold broth micro-dilution method. The bacterial isolates were cultured in nutrient broth for 24 h before the experiment. The bacterial suspensions were then inoculated into microtitre plates, which contained nutrient broth with serial dilutions of worm-cast extract (10, 1, 0.1, 0.01, and 0.001 mg/mL). The positive control was nutrient broth without bacterial inoculation, and the negative control was nutrient broth with bacterial inoculation only. The universal bottles were then incubated for 18–24 h. The MIC values were defined as the lowest concentration of the worm-cast extract in the wells of the microtitre plate that showed no visible turbidity after incubation.

2.7. Minimum bactericidal concentration (MBC)

The method described by Wei et al. [15] was used following a slight modification. MBC was determined by sampling all the macroscopically clear tubes from the MIC assays. The dilutions used were 10 and 0.1 mg/mL. The tolerance of the bacterial strains was observed by using dilutions below and above the known MIC. The suspension was inoculated onto plates of nutrient agar. The MBC of each isolate was reported as the lowest concentration, producing a 99.9% reduction in bacterial viable count in the sub-cultured well contents relative to the initial inoculums.

2.8. Determination of antimitotic activity

The antimitotic potential of the extracts was determined according to an established method [7,16,17]. *Sorghum bicolor* seedlings were grown in 250-mL jars containing distilled water at room temperature. The water in the jars was continuously aerated and changed daily until the roots reached 1.0–1.5 cm.

2.8.1. Incubation of seedlings

Ten seedlings were transferred to a negative control solution (distilled water only), to each test solution, as well as the positive control (cyclophosphamide solution). Extracts and cyclophosphamide were dissolved in 50 mL of distilled water to produce 5 concentrations; 0.1, 1.0, 2.5, 5.0, and 10 mg/mL. Three roots of each incubating sample were collected after 24, 48, and 72 h of incubation to determine root length, mitotic index, and half-maximal effective concentration (EC_{50}).

2.9. Microscopic examination

At 24, 48, and 72 h incubation times, roots were excised 3 mm from the radicle tip. Excised root tips were placed in 1 M HCl for 5 min and rinsed in distilled water, followed by squashing and staining with 2% Giemsa stain. In each radicle tip, the mitotic and total cells were counted in 5–8 fields

(400–500 cells) using a light microscope (× 100) [16]. The mitotic index was calculated as the percentage of meristematic cells undergoing mitosis.

2.10. Statistical analysis

The results are expressed as a mean ± standard deviation (SD). All statistical analyses were performed with the SPSS 17 package. An ANOVA test was used to make comparisons within and between groups. The level of significance was determined in comparison with the control groups. Statistical significance was accepted at $p < 0.05$.

3. Results

The extraction yield with n-hexane, ethanol, and water was 0.054%, 0.059%, and 0.094% w/w, respectively. Table 2 shows the distribution of chemical constituents in the worm-cast extracts; the ethanolic and aqueous extracts had more phyconstituents detected. Meanwhile, flavonoids was not detected in the extracts even after confirmation using different methods.

For antimicrobial screening, *n*-hexane and aqueous extracts showed no potency against the microbial isolates, with results similar to the negative control. However, ethanolic extract showed antibacterial potency against *Streptococcus* sp. and *S. aureus*. Their growth inhibition zone diameter was 14 ± 0.33 mm (compared with a positive control of 11 ± 0.23 mm) and 11 ± 0.45 mm (compared with a positive control of 10 ± 0.69 mm), respectively. The MIC and MBC of the ethanolic extract against *Streptococcus* sp. and *S. aureus* were 1 mg/mL and 0.1 mg/mL, respectively. The aqueous extract showed antifungal activity, with a diameter of 9 ± 0.65 mm and 10 ± 0.41 mm for *A. flavus* and *A. niger*, respectively, while the positive controls were 10 ± 0.89 mm and 12 ± 0.11 mm, respectively. *N*-hexane and ethanolic extracts showed no antifungal potential, as compared to the negative control.

The antimitotic assay showed that all the tested worm-cast extracts significantly ($p < 0.05$) retarded and inhibited mitotic activity in the root tips of *S. bicolor*. The decrease in mitotic activity was proportional to the increasing concentrations of the extracts as well as the incubation time. The increase in radicle length after 24 h incubation was affected by all treatments in a dose-dependent manner (Table 3). A similar trend was observed at 48 h (Table 4) and 72 h (Table 5), but deterioration of roots was seen on the root tips treated with cyclophosphamide after 72 h incubation.

The values of mitotic index determined in meristematic cells of the radicle tips collected at various time intervals, i.e. at 24 h (Table 6), 48 h (Table 7), and 72 h (Table 8), were found to be similar. Ethanolic and aqueous extracts of the worm-casts produced a significant decrease in mitotic index, which was dependent on both concentration and duration of exposure. Similarly, the effect of cyclophosphamide on root tips did not differ significantly from that of worm-cast extracts, while the mitotic index of untreated root tips was significantly higher than that of treated root tips ($p < 0.05$). The obtained EC_{50} values for anti-mitotic activity were in the order: ethanolic extract > cyclophosphamide > aqueous extract (5.937 > 2.576 > 1.767).

Furthermore, the micrographs show that the treated healthy cells of *S. bicolor* (Fig. 1b) exhibited more pyknotic nuclei (Fig. 1c), indicating mitotic arrest for both the positive control (cyclophosphamide) and the worm-cast extracts. At high concentrations of

Table 2. Qualitative analysis of phytochemicals in n-hexane, ethanolic, and aqueous extracts of worm-casts of Hyperiodrilus africanus.

Test type	Test name	N-hexane extract	Ethanolic extract	Aqueous extract
Alkaloids	Mayer test	−	++	+
Saponins	Froth test	−	−	−
Anthraquinones	Borntrager test	−	+	+
Coumarins	Fluorescence test	−	+	+
Sterols &terpenes	Liebermann–Burchard test	−	−	−
Steroids	Liebermann–Burchard test	+	+	+
Terpenoids	Salkowski test	−	+	−
Flavonoids	Ammonium chloride test	−	−	−
	Potassium hydroxide test	−	−	−
	Ammonia test	−	−	−
	Ethyl acetate test	−	−	−
Tannins	Braymer test	−	+	+
Phlobatannins	Hydrochloric acid test	−	−	−
Cardiac glycosides	Keller–Killani test	−	+	+
Phenols	Ferric chloride test	−	+	−

Table 3. Effect of extracts of over-seasoned worm-casts of Hyperiodrilus africanus on root length of Sorghum bicolor after 24-h incubation.

Concentration of incubating solution (mg/ml)	Root length (cm)			
	negative control (water)	positive control (cyclophos-phamide)	aqueous extract solution	ethanolic extract solution
0.1	2.3 ± 0.076^{a}	2.2 ± 0.071^{a}	2.1 ± 0.070^{a}	2.2 ± 0.078^{a}
1.0	2.3 ± 0.076^{a}	2.0 ± 0.070^{b}	2.0 ± 0.071^{b}	2.1 ± 0.565^{b}
2.5	2.3 ± 0.076^{a}	1.9 ± 0.141^{b}	2.1 ± 0.078^{bc}	2.0 ± 0.454^{c}
5.0	2.3 ± 0.076^{a}	1.8 ± 0.088^{b}	1.9 ± 0.065^{bc}	1.8 ± 0.077^{cd}
10.0	2.3 ± 0.076a	1.6 ± 0.076^{c}	1.7 ± 0.089^{c}	1.7 ± 0.565^{d}

Table 4. Effect of extracts of over-seasoned worm-casts of Hyperiodrilus africanus on root length of Sorghum bicolor after 48-h incubation.

Concentration of incubating solution (mg/mL)	Root length (cm)			
	negative control (water)	positive control (cyclophos-phamide)	aqueous extract solution	ethanolic extract solution
0.1	3.5 ± 0.985^{a}	3.1 ± 0.675^{a}	3.0 ± 0.034^{a}	2.9 ± 0.075^{a}
1.0	3.5 ± 0.985^{a}	2.8 ± 0.676^{b}	2.8 ± 0.078^{b}	2.7 ± 0.098^{b}
2.5	3.5 ± 0.985^{a}	2.7 ± 0.456^{c}	2.6 ± 0.374^{b}	2.4 ± 0.087^{c}
5.0	3.5 ± 0.985^{a}	1.9 ± 0.454^{d}	2.5 ± 0.845^{c}	2.2 ± 0.078^{d}
10.0	3.5 ± 0.985^{a}	1.7 ± 0.707^{e}	2.3 ± 0.785^{d}	2.0 ± 0.076^{e}

Values are means of duplicates ± SD. Within columns, different superscripts denote significant differences ($p < 0.05$). Linear regression of extract concentration vs. root length had p values of 0.033 and 0.021 for aqueous and ethanolic extracts, respectively.

Table 5. Effect of extracts of over-seasoned worm-casts of Hyperiodrilus africanus on root length of Sorghum bicolor after 72-h incubation.

Concentration of incubating solution (mg/mL)	Root length (cm)			
	negative control (water)	positive control (cyclophos-phamide)	aqueous extract solution	ethanolic extract solution
0.1	4.1 ± 0.878^{a}	3.4 ± 0.078^{a}	3.3 ± 0.067^{a}	3.1 ± 0.784^{a}
1.0	4.1 ± 0.878^{a}	3.0 ± 0.076^{b}	3.1 ± 0.096^{b}	2.8 ± 0.213^{b}
2.5	4.1 ± 0.878^{a}	*2.7 ± 0.068^{c}	2.8 ± 0.989^{c}	2.6 ± 0.414b^{c}
5.0	4.1 ± 0.878^{a}	*1.6 ± 0.087^{d}	2.6 ± 0.045^{d}	2.4 ± 0.071^{c}
10.0	4.1 ± 0.878^{a}	*1.2 ± 0.087^{e}	2.4 ± 0.099^{e}	2.1 ± 0.089^{d}

Asterisks indicate root decay. Values are means of duplicates ± SD. Within columns, different superscripts denote significant differences ($p < 0.05$). Linear regression of extract concentration vs. root length had p values of 0.058 and 0.013 for aqueous and ethanolic extracts, respectively.

cyclophosphamide (positive control) and ethanolic extract, the onset of cellular architecture distortion was obvious (Fig. 1a).

4. Discussion

Empirical insights on the medicinal relevance of over-seasoned worm-casts are scarce. Most studies of Nigerian earthworms only show the agricultural relevance of worm-casts, with little attention paid to their medicinal benefits [4,18,19]. Fresh worm-casts of *H. africanus* are characterized by high enzymatic activity, high antimicrobial properties, and high levels of calcium, magnesium, sodium, potassium, and phosphorus [4,6,20]. They have also been

Table 6. Effect of extracts of over-seasoned worm-casts of Hyperiodrilus africanus on the mitotic index of Sorghum bicolor cells after 24-h incubation.

Concentration of incubating solution (mg/mL)	Mitotic index (%)			
	negative control (water)	positive control (cyclophos-phamide)	aqueous extract solution	ethanolic extract solution
0.1	94.89 ± 2.23^{a}	90.45 ± 2.34^{a}	91.43 ± 3.23^{a}	92.11 ± 1.32^{a}
1.0	94.89 ± 2.23^{a}	80.73 ± 4.23^{b}	88.03 ± 3.45^{ab}	86.49 ± 2.45^{b}
2.5	94.89 ± 2.23^{a}	64.23 ± 1.34^{c}	76.32 ± 3.22^{b}	79.25 ± 2.43^{c}
5.0	94.89 ± 2.23^{a}	58.62 ± 5.32^{d}	73.17 ± 3.12^{c}	72.97 ± .23^{d}
10.0	94.89 ± 2.23^{a}	50.43 ± 2.43^{e}	70.03 ± 5.67^{c}	60.34 ± 1.23^{e}

Values are means of duplicates ± SD. Within columns, different superscripts denote significant differences ($p < 0.05$). Linear regression of extract concentration vs. mitotic index had p values of 0.008 and 0.002 for aqueous and ethanolic extracts, respectively.

Table 7. Effect of extracts of over-seasoned worm-casts of Hyperiodrilus africanus on the mitotic index of Sorghum bicolor cells after 48-h incubation.

Concentration of incubating solution (mg/mL)	Mitotic index (%)			
	negative control (water)	positive control (cyclophos-phamide)	aqueous extract solution	ethanolic extract solution
0.1	97.12 ± 2.11[a]	85.71 ± 3.45[a]	80.55 ± 1.23[a]	69.64 ± 1.43[a]
1.0	97.12 ± 2.11[a]	73.91 ± 2.12[a]	74.07 ± 3.12[a]	63.16 ± 4.20[b]
2.5	97.12 ± 2.11[a]	66.67 ± 2.34[b]	68.63 ± 3.11[b]	60.53 ± 4.22[c]
5.0	97.12 ± 2.11[a]	*38.71 ± 2.12[c]	62.41 ± 4.23[b]	54.54 ± 5.34[d]
10.0	97.12 ± 2.11[a]	*41.67 ± 4.12[d]	58.85 ± 5.21[c]	*47.62 ± 1.32[e]

Asterisks indicate cell morphology damage. Values are means of duplicates ± SD. Within columns, different superscripts denote significant differences ($p < 0.05$). Linear regression of extract concentration vs. mitotic index had p values of 0.074 and 0.007 for aqueous and ethanolic extracts, respectively.

Table 8. Effect of Hyperiodrilus africanus over-seasoned cast extracts on the mitotic index of Sorghum bicolor after 72 h incubation.

Concentration of incubating solution (mg/mL)	Mitotic index (%)			
	negative control (water)	positive control (cyclophos-phamide)	aqueous extract solution	ethanolic extract solution
0.1	98.78 ± 3.43[a]	76.34 ± 3.12[a]	72.54 ± 4.12[a]	58.30 ± 4.23[a]
1.0	98.78 ± 3.43[a]	66.67 ± 2.33[b]	61.11 ± 1.20[a]	53.02 ± 2.34[a]
2.5	98.78 ± 3.43[a]	61.05 ± 2.40[c]	55.55 ± 4.10[b]	50.23 ± 2.54[b]
5.0	98.78 ± 3.43[a]	*40.48 ± 3.11[d]	50.85 ± 2.40[c]	*44.54 ± 5.55[bc]
10.0	98.78 ± 3.43[a]	*36.78 ± 4.12[e]	54.01 ± 1.23[d]	*41.34 ± 1.23[c]

Asterisks indicate cell morphology damage. Values are means of duplicates ± SD. Within columns, different superscripts denote significant differences ($p < 0.05$). Linear regression of extract concentration vs. mitotic index had p values of 0.044 and 0.020 for aqueous and ethanolic extracts, respectively.

reported to break seed dormancy and efficiently promote germination [21].

Screening of the extracts for chemical constituents revealed that the ethanolic and aqueous extracts had more chemical constituents than *n*-hexane extracts [22]. Many phytochemicals are currently believed to serve as protective agents for plants against invading enemies, so earthworms can derive

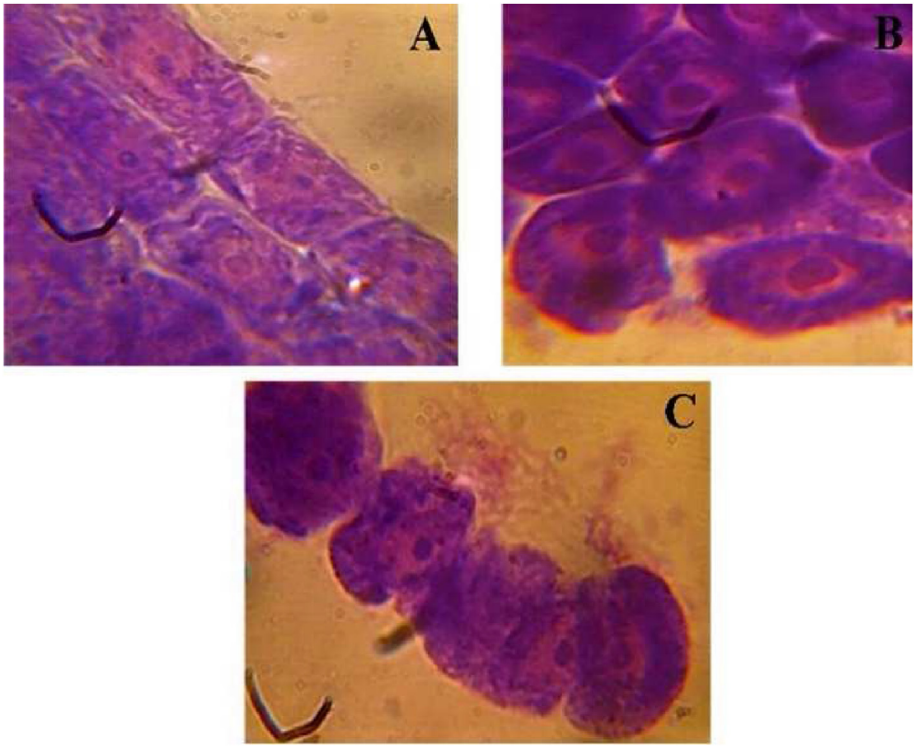

Fig. 1. Cells under different conditions: (a) cells at the onset of cell morphology damage; (b) healthy cells; (c) cells with pyknotic nuclei (Scale bar – 100 μM).

secondary protective benefits from these phytochemicals when they are readily available in aqueous forms in the worm-casts [23]. Most of the phytochemicals identified in the earthworm cast had been previously implicated for a variety of medicinal value [24,25]. For example, tannins, phenols steroids, anthraquinones, and coumarins have been shown to possess medicinal properties such as antioxidant, anti-inflammatory, anti-parasitic, and antimicrobial among others [26–31].

In essence, the antimicrobial properties of the over-seasoned worm-casts of *H. africanus* may be attributable to the synergistic effects of the various bioactive compounds, such as the tannins, phenols, steroids, alkaloids, anthraquinones, and coumarins in the extracts of the worm casts. Together, the findings in the present study support the already established use of the worm-casts of *H. africanus* as a traditional remedy for various enteric conditions: the worm-casts are ground in water, sieved, and the filtrate is drunk by rural dwellers to treat enteritis [32].

Furthermore, the antimitotic effect of the worm-cast extract may be a result of some phytochemicals and other unidentified bioactive components present in the casts [33]. Some of the phytochemicals such as tannin and phenols, identified in the extracts of the worm casts have anti-cancer properties [34]. The ability of the worm-cast extracts to induce the development of pyknosis in healthy cells suggests that the worm-cast extract causes the condensation of chromatin in the nucleus by its effect on microtubule dynamics [6]. The antimitotic activity exhibited by the worm-cast extracts compared favorably to the reference drug, cyclophosphamide.

5. Conclusion

In conclusion, our findings provide evidence supporting the promising candidature of over-seasoned worm-casts of *H. africanus* as an alternative source of cytotoxic agents, which may find relevance in cancer therapy and other related ailments. Also, the ethanolic extract showed antibacterial activity against *Streptococcus* sp. and *S. aureus,* while the aqueous extract showed antifungal activity against *A. flavus* and *A. niger*. Taken together, the present findings provide evidence, lending credence to the antimicrobial and cytotoxic potential of over-seasoned casts of *H. africanus*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We appreciate the help of the laboratory staff at the Department of Biochemistry, Landmark University, Omu-Aran, Nigeria.

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