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Evaluation of antifungal and antioxidant activities of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents

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Abstract. *This study evaluated the antifungal and antioxidant activities of earthworm (Perionyx excavatus) powder extracts using different solvents including 80% ethanol, 50% ethanol, and distilled water. The extraction efficiencies ranged from 18.5 to 21.2%, while total protein contents ranged from 64.8 to 67.5%. Notably, the aqueous extract exhibited the highest values in both extraction efficiency and total protein content. Thin-layer chromatography analysis revealed the presence of amino acids and peptides with R_f values ranging from 0.42 to 0.65. Fourier-transform infrared spectroscopy spectra displayed characteristic peaks associated with protein structures. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated protein compositions primarily below 50 kDa molecular weight in the extracts, particularly with the 80% ethanol extract predominantly consisting of proteins below 30 kDa. Antifungal tests against Candida albicans demonstrated the highest efficacy with 80% ethanol extract, exhibiting an inhibition zone diameter of 13.3 mm and a minimum inhibitory concentration of 75 mg/mL. Additionally, the extracts showed DPPH and ABTS radical scavenging activities, with 80% ethanol extract displaying the highest antioxidant potential with IC_{50} values of 231.3 μg/mL for DPPH assay and 208.9 μg/mL for ABTS assay. In conclusion, earthworm powder extracts exhibited significant biological activities, rendering them promising candidates for pharmaceutical and cosmetic applications.*

Keywords: *Earthworm, Perionyx excavatus, Candida albicans, DPPH, ABTS*

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Оценка противогрибковой и антиоксидантной активности экстрактов, полученных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей

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Аннотация. *В данном исследовании были оценены противогрибковые и антиоксидантные активности экстрактов из порошка дождевого червя (Perionyx excavatus), приготовленных с использованием различных растворителей,*

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включая 80%-й этанол, 50%-й этанол и дистиллированную воду. Эффективность экстракции варьировалась от 18,5 до 21,2%, в то время как содержание общего белка колебалось от 64,8 до 67,5%. Примечательно, что водный экстракт продемонстрировал наивысшие значения как по эффективности экстракции, так и по содержанию белка. Анализ методом тонкослойной хроматографии выявил присутствие аминокислот и пептидов с R_f-значениями от 0,42 до 0,65. Спектры инфракрасной спектроскопии с преобразованием Фурье показали характерные пики, связанные со структурой белков. Электрофорез в полиакриламидном геле с додецилсульфатом натрия указал на наличие белков преимущественно с молекулярной массой ниже 50 кДа в экстрактах, особенно в экстракте с 80%-м этанолом, который в основном содержал белки с молекулярной массой ниже 30 кДа. Противогрибковые тесты против Candida albicans показали наивысшую эффективность у экстракта с 80%-м этанолом с диаметром зоны ингибирования 13.3 мм и минимальной ингибирующей концентрацией 75 мг/мл. Кроме того, экстракты продемонстрировали активность в отношении улавливания радикалов DPPH и ABTS, причем экстракт с 80%-м этанолом показал наивысший антиоксидантный потенциал со значениями IC_{50} 231,3 мкг/мл для DPPH и 208,9 мкг/мл для ABTS. В заключение необходимо отметить, что экстракты из порошка дождевого червя проявили значительную биологическую активность, что делает их перспективными кандидатами для применения в фармацевтике и косметологии.

Ключевые слова: *дождевой червь, Perionyx excavatus, Candida albicans, DPPH, ABTS*

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INTRODUCTION

In Vietnam, a rich diversity of flora and fauna has long been utilized in traditional medicine to treat various ailments, owing to their inherent therapeutic properties[1] [1]. Despite remarkable advancements in modern healthcare, a significant portion of the population continues to rely on traditional remedies derived from plants and animals[1] [2]. As interest grows in integrating traditional and modern medicine, there is a burgeoning trend in exploring the beneficial properties of natural products. Many natural sources have demonstrated significant biological activities, including antimicrobial and antioxidant properties, crucial for combating infectious diseases and oxidative stress-related disorders [3]. Moreover, natural products often exhibit fewer adverse effects compared to synthetic alternatives [4]. Of particular interest is the study of earthworms, a traditional medicinal resource in Vietnam, which has garnered attention for its potential therapeutic applications.

Earthworms are invertebrates that have been widely used medicinally in traditional medicine for centuries [5]. Extraction and utilization of bioactive compounds from earthworms have been practiced globally, particularly in Asian countries such as India, South Korea, China, and Vietnam [5, 6]. Earthworms are believed to enhance health and prevent various diseases. Earthworm proteins possess high nutritional value and can serve as high-quality animal-origin protein supplements for nutrition enrichment [7, 8]. Furthermore, earthworm species such as *Lampito*, *Eudrilus*, *Perionyx*, *Eisenia*, and *Metaphire* have demonstrated various preventive and therapeutic effects. Numerous studies have indicated that earthworm extracts contain a plethora of bioactive molecules exhibiting diverse biological functions, including antimicrobial [9–13], wound healing promotion [14, 15], anti-inflammatory [16–18], and antioxidant activities [19–21]. They have shown inhibitory effects on cancer cells such as MCF-7 breast cancer cells, prostate cancer cells, and colorectal cancer cells [22–25].

Perionyx excavatus is one of the most common earthworm species in Vietnam, particularly in southern provinces such as Ho Chi Minh City, Dong Nai, and Ninh Thuan [26]. This species possesses both nutritional value and high biological activity [27]. However, research on the antimicrobial and antioxidant activities of this earthworm species in Vietnam remains relatively limited. Therefore, in this study, we conducted extractions from *P. excavatus* using different solvents and evaluated their antifungal and antioxidant activities, laying the groundwork for the application of earthworms in the pharmaceutical and cosmetic industries.

MATERIALS AND METHODS

Earthworm samples. Living earthworms (*P. excavatus*), aged 7–8 weeks, were collected from a farm located in Cu Chi district, Ho Chi Minh City, Vietnam. Upon collection, they were promptly transported to the Biotechnology Center of Ho Chi Minh City, Vietnam. The earthworms underwent processing following the method outlined by Azmi et al. [28], with some modifications. Initially, the earthworms were washed under running water to clean the dirt from the body surface. Subsequently, they were immersed in 1.0% NaCl solution for 15 min, followed by a 0.3% citric acid solution for 20 min. Following this treatment, the earthworms were freeze-dried to yield earthworm powder, which was then stored at 40 °C.

Preparation of ethanol extract (E80 and E50). 30 g of earthworm powder was placed into a 500 mL beaker and mixed with either 300 mL of 80% ethanol (*E*80) or 50% ethanol (*E*50) at a 1:10 (w/v). The mixture was stirred at

[1] Nguyen D.N.V., Nguyen T. An overview of the use of plants and animals in traditional medicine systems in Viet Nam: a Traffic Southeast Asia report. Ha Noi: Traffic Southeast Asia, 2008. 96 p.

room temperature for 8 h and filtered through the Whatman filter paper. The ethanol solvent was then evaporated. The resulting solutions were combined with 400 mL of hexane and stirred for 60 min at room temperature. The hexane solvent was removed, and the mixture was filtered through a 0.22 μm membrane, and then freeze-dried to obtain the extract powder (*E*80 and *E*50).

Preparation of aqueous extract (W). 20 g of earthworm powder was placed into a 500 mL beaker and mixed with 200 mL of distilled water. The mixture was shaken vigorously and broken using an ultrasonic device (Sonicator-Q700) with intensity fluctuating at 50% for 10 min. Subsequently, the sample was heated to boiling for 60 min on a stirrer and filtered using Whatman filter paper. The resulting filtrate was combined with 300 mL of hexane solvent and stirred for an additional 60 min. After removing the hexane, the solution was filtered through a 0.22 μm membrane. The filtered solution was then freeze-dried to obtain the extract powder (*W*).

Determination of extraction efficiency. The extraction efficiency *EE* of each earthworm extract (*E*80, *E*50, and *W*) was calculated based on the formula: % $EE = W_1/W_2 \times 100$, where W_1 is the weight of the obtained extract powder, g, and W_2 is the weight of the sample initial, g.

Determination of protein content. The total protein content in the earthworm extracts was assessed using the Kjeldahl method [29]. 1 g of each earthworm extract (*E*80, *E*50, and *W*) was added to a Kjeldahl digestion flask containing 25 mL of concentrated H_2SO_4, along with a catalyst (9 g of K_2SO_4 and 1 g of $CuSO_4 \cdot 5H_2O$). After 2.5 h of digestion in a unit with electrical heat and fume removal, the mixture was allowed to cool to room temperature. Subsequently, 80 mL of NaOH base was added to the flask. Following distillation, the collected ammonia underwent titration with a standardized solution of HCl to determine its concentration. The nitrogen content in the sample was then calculated based on the volume and concentration of the acid used in the titration. Since proteins typically contain about 16% nitrogen by weight, the total protein content of the sample was calculated using a conversion factor of 6.25.

Thin-layer chromatography analysis. The earthworm extracts (*E*80, *E*50, and *W*) were subjected to thin-layer chromatography (TLC) analysis by dissolving them in ethanol and applying them onto a TLC plate measuring 7×10 cm, coated with silica gel 60 F254 (Merck, Darmstadt, Germany). A mobile phase consisting of a mixture of *n*-butanol, acetic acid, and water in the ratio 5:1:4 was used for chromatographic separation. Subsequently, the TLC plate was sprayed with a solution of ninhydrin (1.5 g ninhydrin in 100 mL ethanol) and then dried at 45 °C for 10 min. Observation of the resulting chromatogram allowed for the determination of both the color and the migration coefficient R_f of the substance traces.

Fourier-transform infrared spectroscopy analysis. Fourier-transform infrared spectroscopy (FTIR) analysis was conducted using a method outlined by Ramya et al. [30]. A mixture comprising 10 mg of earthworm extracts (*E*80, *E*50, and *W*) and 100 mg of dried potassium bromide (KBr) was prepared. This mixture was then pelletized under compression at a diameter of 3 mm and a thickness of 1 mm. Subsequently, the absorbance spectra were obtained using a Bio-Rad FTIR-40-model spectrometer from the USA. FTIR analysis relies on the principle that different components within the sample absorb infrared radiation at distinct frequencies.

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis analysis. The molecular weights of earthworm proteins were determined using sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) according to the method outlined by Hua et al. [31]. Initially, the earthworm protein samples (*E*80, *E*50, and *W*) were dissolved in a buffer solution consisting of 20 mM Tris-HCl pH 8.0, 8 M urea, 2% sodium dodecyl sulfate (SDS), and 2% 2-mercaptoethanol. Following dissolution, the samples underwent centrifugation at 5000 rpm for 5 min. Subsequently, the protein sample was mixed in a 1:1 ratio (v/v) with a buffer solution containing 0.5 M Tris-HCl pH 6.8, 10% SDS, glycerol at 100%, bromophenol blue, and 2% 2-mercaptoethanol. This mixture was then boiled for 5 min to denature the proteins and ensure uniform binding of SDS. For the electrophoretic separation of proteins, a 16% polyacrylamide resolving gel was prepared, topped by a 5% stacking gel. The prepared protein samples were loaded onto the gel and subjected to electrophoresis under denaturing conditions. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250, allowing visualization of the separated protein bands. Excessive stain was removed by destaining the gel in a solution containing 7% acetic acid and 25% methanol until protein bands became clearly visible against the destained background.

Antifungal assay. The antifungal activity of earthworm extracts (*E*80, *E*50, and *W*) against *Candida albicans* ATCC 10231 was assessed using the agar well diffusion method, as described by Bhorgin and Uma [12], with some modifications. *C. albicans* ATCC 10231 was provided by the Biotechnology Center of Ho Chi Minh City, Vietnam. Initially, *C. albicans* were inoculated onto Mueller-Hinton Agar (MHA) plates with a concentration of 10^6 CFU/mL, and incubated at 37 °C for 48 h. Diluted earthworm extracts were dispensed into wells on the agar plates. Distilled water served as the negative control, while gentamicin was employed as the positive control. Following incubation at 37 °C for 24 h, the inhibition zones were measured to evaluate antifungal activity.

Furthermore, the minimum inhibitory concentration (MIC) of the earthworm extracts (*E*80, *E*50, and *W*) was determined by a broth microdilution assay using sterile 96-well flat-bottom microtiter plates as described by Thin et al. [32]. Initially, *C. albicans* was cultured in Tryptic Soy Broth (TSB) at 37 °C for 24 h. Subsequently, the culture was diluted to a concentration of 10^6 CFU/mL using Mueller-Hinton Broth (MHB) via the McFarland method. 50 μL of the earthworm extracts at varying concentrations were mixed with an equal volume of fungal suspension in each well and incubated at 35 °C for 24 h. Following this, 30 μL of 0.02% resazurin was added to each well and further incubated at 35 °C for 30 min. The MIC was determined as the lowest concentration of the extract at which the resazurin reagent did not turn pink, indicating inhibition of fungal growth.

Antioxidant assay. The antioxidant activity of earthworm extracts (*E*80, *E*50, and *W*) was assessed through DPPH and ABTS methods, following established protocols [33, 34]. For the DPPH assay, various concentrations of

earthworm extracts were prepared in methanol. Each sample (0.9 mL) was mixed with 4 mL of 0.1 mM DPPH solution in methanol, followed by incubation at 25 °C for 30 min in the dark. Absorbances were then measured at 517 nm using a microplate reader (VersaMax, Molecular Devices). Vitamin C served as a positive control. The percentage of DPPH radical-scavenging activity was calculated using the formula: % inhibition = $(1\text{-}A_T/A_C)\times100$, where A_C represents the absorbance of the control sample and A_T represents the absorbance of the test samples.

In the ABTS assay, the ABTS radical cation was prepared by reacting ABTS solution (7 mM) in methanol with potassium persulfate (2.45 mM) at a 1:1 ratio. The resulting ABTS stock was left in the dark at 24 °C for 16 h before use. This stock solution was then diluted with methanol to achieve an absorbance of 0.70 (±0.02) at 734 nm. Earthworm extracts, dissolved in methanol at various concentrations, were mixed with the ABTS stock solution (5 mL) and incubated in the dark for 15 min. Absorbance was measured at 734 nm using a microplate reader (VersaMax, Molecular Devices). Vitamin C was used as the positive control. The percentage of ABTS radical scavenging activity was calculated similarly to the DPPH assay.

The IC_{50} value, representing the concentration of the sample causing a 50% reduction in the initial DPPH or ABTS concentration, was determined from the concentration curve of the test extract against the percentage of radical scavenging inhibition obtained through linear regression analysis.

Statistical analysis. All analyses were carried out in triplicate, and the results were calculated as the mean ± standard deviation (*SD*) using Microsoft Excel 2016.

RESULTS

Extraction efficiency and total protein content of earthworm extracts. The extraction efficiency and total protein content of earthworm powder extracts using different solvents were evaluated. As shown in Table 1, the extraction efficiencies of the three samples ranged from 18.5 to 21.2%, while the total protein contents ranged from 64.8 to 67.5%. The highest extraction efficiency was observed with an aqueous extract, yielding 21.2%, while the lowest efficiency was obtained with 80% ethanol extract at 18.5%. Similarly, regarding protein content, the highest percentage was detected in the aqueous extract (67.5%), followed closely by the 50% ethanol extract (64.8%), and the lowest was observed in the 80% ethanol extract (66.8%).

Table 1. Extraction efficiency and total protein content of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents

Таблица 1. Эффективность экстракции и общее содержание белка в экстрактах, приготовленных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей

Sample extract	Extraction efficiency, %	Total protein content, %
*E*80	18.5±1.7	66.8±0.35
*E*50	19.7±1.1	64.8±0.91
W	21.2±1.2	67.5±0.66

Note. Results are means of three measurements ± standard deviation (±*SD*).

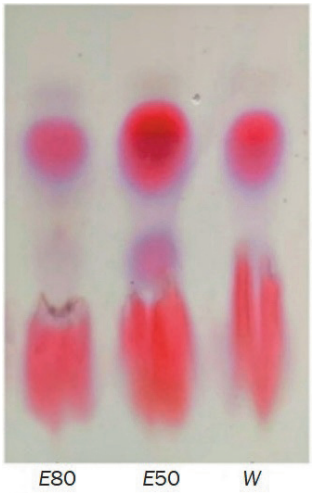

Fig. 1. Thin-layer chromatography of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents

Рис. 1. Тонкослойная хроматография экстрактов, полученных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей

TLC analysis of earthworm extracts. TLC results of earthworm powder extracts using different solvents are presented in Fig. 1. Pinkish-purple spots with R_f values ranging from 0.42 to 0.65 on the TLC plate indicated the presence of amino acids and peptides.

Fourier-transform infrared spectroscopy analysis of earthworm extracts. FTIR spectroscopy analysis of earthworm powder extracts using different solvents revealed distinctive peaks associated with proteins (Fig. 2). These included characteristic absorption bands corresponding to various protein functional groups. Notably, peaks at 3400 cm^{-1} were attributed to NH group valence oscillations, indicative of peptide bonds. The presence of proteinaceous materials was further supported by the absorption band at 1643 cm^{-1}, corresponding to amide I oscillation. Oscillations within the amide II region (1407–1514 cm^{-1}) were primarily attributed to valence oscillations of C-N and C=O groups, along with contributions from other functional groups such as C≡N and CCN. The spectra also revealed the presence of amide III within the range of 1049–1218 cm^{-1}, indicating protein structures through N-H bending oscillations and deformations of C-H bonds. Additionally, absorption bands at 513–649 cm^{-1} were attributed to C=O bend oscillations outside the plane, further supporting the presence of protein components. Overall, the FTIR spectra confirmed the presence of characteristic peaks associated with protein amides I, II, and III in the earthworm powder extracts.

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis analysis of earthworm extracts. Based on the SDS-PAGE analysis of proteins in earthworm powder extract using different solvents, the results revealed distinct patterns in the protein composition (Fig. 3). In general, proteins present in extracts from earthworm powder mainly included fractions with molecular weights below 50 kDa. In particular, the extraction process using 80% ethanol solvent mainly resulted in proteins with molecular weights below 30 kDa.

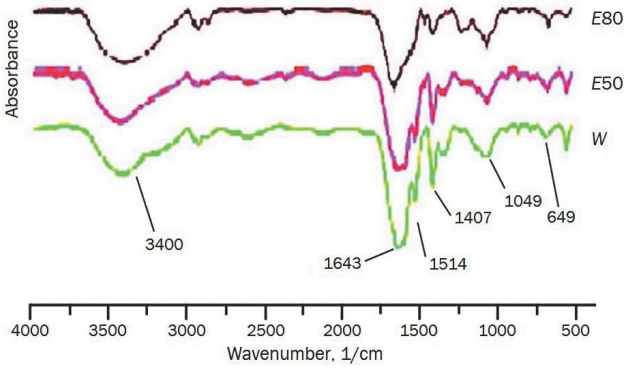

Fig. 2. Fourier-transform infrared spectra of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents

Рис. 2. Инфракрасные Фурье-спектры экстрактов, полученных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей

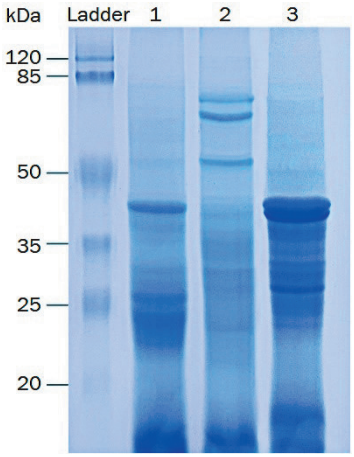

Fig. 3. Sodium dodecyl sulfate – polyacrylamide gel electrophoresis analysis of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents: 1 – 50% ethanol extract; 2 – aqueous extract; 3 – 80% ethanol extract

Рис. 3. Выполненный при помощи электрофореза в полиакриламидном геле с додецилсульфатом натрия анализ экстрактов, полученных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей: 1 – 50%-й экстракт этанола; 2 – водный экстракт; 3 – 80%-й экстракт этанола

Antifungal activity of earthworm extracts. The antifungal activity of earthworm powder extracts using different solvents against *C. albicans* is presented in Table 2. For the agar well diffusion assay, the 80% ethanol extract exhibited the highest efficacy, producing a maximum inhibition zone diameter of 13.3±1.50 mm. Following this, the 50% ethanol extract showed moderate activity with a maximum inhibition zone diameter of 6.3±0.28 mm, while the aqueous extract exhibited the lowest efficacy with a maximum inhibition zone diameter of 5.3±0.25 mm (Fig. 4). Additionally, the MIC data revealed the inhibitory effect of earthworm powder extracts on *C. albicans* growth, with MIC values of 75 mg/mL for 80% ethanol extract, 150 mg/mL for 50% ethanol extract, and 250 mg/mL for aqueous extract (see Table 2).

Table 2. Antifungal activity of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents

Таблица 2. Противогрибковая активность экстрактов, полученных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей

Fungal strain	*E*80	*E*50	*W*	Gentamicin
Candida albicans	Diameter of inhibition zones, mm			
	13.3±1.50	6.3±0.28	5.3±0.25	20.6±2.08
	Value of the minimum inhibitory concentration, mg/mL			
	75	150	250	–

Note. Results are means of three measurements ±*SD*

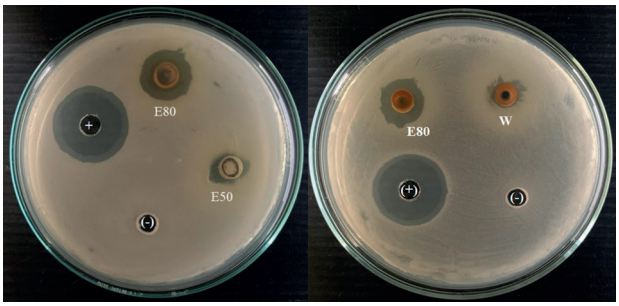

Fig. 4. Antifungal effect of extracts prepared from earthworm (*Perionyx excavatus*) against *Candida albicans* using the agar well diffusion method

Рис. 4. Противогрибковая активность экстрактов, приготовленных из дождевого червя (*Perionyx excavatus*), по отношению к *Candida albicans*, выявленная методом луночной диффузии в агаре

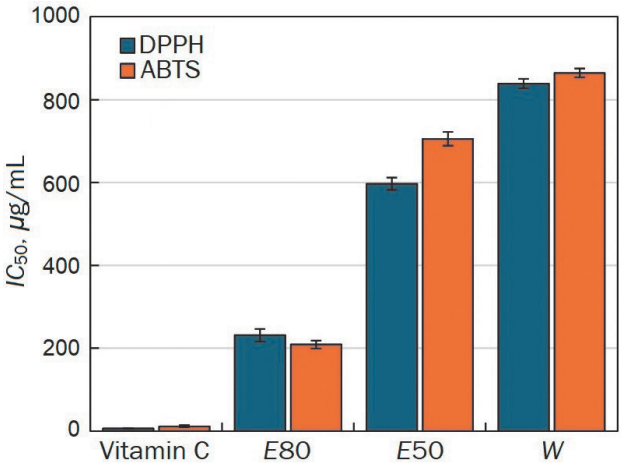

Fig. 5. Antioxidant activity (DPPH and ABTS) of extracts prepared from earthworm (*Perionyx excavatus*) using different solvents (results are means of three measurements ±*SD*)

Рис. 5. Антиоксидантная активность (DPPH и ABTS) экстрактов, приготовленных из дождевого червя (*Perionyx excavatus*) с использованием различных растворителей (результаты представляют собой среднее значение трех измерений ±*SD*)

Antioxidant activity of earthworm extracts. The results of the DPPH and ABTS scavenging activity of earthworm

powder extracts using different solvents are shown in Fig. 5. The IC_{50} values for inhibiting the DPPH radical were found to be 231.3±15.1 μg/mL for the 80% ethanol extract, 597.1±14.9 μg/mL for the 50% ethanol extract, and 838.7±11.6 μg/mL for the aqueous extract. In terms of ABTS free radical scavenging activity, the 80% ethanol extract showed an IC_{50} value of 208.9±9.6 μg mL, while the aqueous extract exhibited an IC_{50} value of 864.5±10.6 μg/mL, which was higher than the IC_{50} value of 705.5±16.5 μg/mL observed for the 50% ethanol extract. As a positive control, Vitamin C demonstrated significant antioxidant activity with IC_{50} values of 6.1±0.3 μg/mL for the DPPH assay and 11.7±2.6 μg/mL for the ABTS assay.

DISCUSSION

In recent years, there has been significant attention on researching the biological activity of natural products for potential applications across various fields [4]. This interest stems from the recognition of the vast chemical diversity present in natural sources, offering a rich repository of potentially bioactive compounds [35]. In this study, we focused on extracting earthworm (*P. excavatus*) using different solvents: 80% ethanol, 50% ethanol, and distilled water. Our results indicate that distilled water as a solvent yielded the highest extraction efficiency at 21.2% (see Table 1). This may be because water is a polar solvent, which facilitates the dissolution of polar compounds present in earthworm powders [36]. Many bioactive compounds, such as phenolics, flavonoids, and polysaccharides, are hydrophilic and thus more readily soluble in water. On the other hand, ethanol, while also capable of dissolving polar compounds, may not be as efficient as water due to its partial non-polar nature [37]. This observation underscores the importance of solvent selection in optimizing extraction protocols for natural products. Previous studies have similarly demonstrated that extraction efficiency is influenced by various factors, among which the solvent plays a critical role [38, 39]. Factors such as solvent polarity, viscosity, and ability to disrupt cellular structures can all impact extraction efficiency [37].

Earthworms are known to be rich sources of protein, making their quantification crucial for evaluating their nutritional composition and assessing their suitability for different purposes [7]. In this study, we found that the total protein content of earthworm extracts varied depending on the solvent used for extraction, ranging from 64.8 to 67.5% (see Table 1). This variability underscores the importance of solvent selection in maximizing protein yield from earthworm tissues. Moreover, our results indicate that aqueous extraction yielded the highest total protein content (67.5%) among the solvents tested. As emphasized above, the higher protein content obtained with distilled water as a solvent can be since water is a polar solvent, which is particularly effective in the extraction of polar and hydrophilic compounds such as proteins [36]. The affinity of water molecules for protein molecules facilitates their solubilization and extraction from earthworm tissues. In contrast, ethanol, while capable of extracting proteins, may not be as efficient due to differences in solubility and selectivity [37]. Particularly at higher concentrations, ethanol may precipitate proteins or interfere with their solubility, resulting in lower overall protein extraction efficiency [37]. Solvent effects on the total protein content of extracts have also been reported previously [40, 41].

The combination of TLC, FTIR, and SDS-PAGE analyses provided comprehensive confirmation of the presence of proteins in the earthworm extracts obtained using different solvents. TLC analysis revealed pinkish-purple spots with R_f values ranging from 0.42 to 0.65, indicative of the presence of amino acids and peptides, fundamental components of proteins (see Fig. 1). This aligns with previous studies demonstrating that R_f values in the range of 0.2 to 0.8 correspond to amino acids and peptides [42]. FTIR spectroscopy further supported protein identification by showing characteristic peaks corresponding to amides I, II, and III in the earthworm extracts (see Fig. 2). These amide peaks are well-established indicators of protein presence in FTIR spectra, closely related to protein structure and conformation [30, 43]. Moreover, SDS-PAGE analysis confirmed the presence of proteins in the earthworm extracts and provided insights into their molecular weights. Specifically, proteins with molecular weights less than 50 kDa were observed, with the 80% ethanol extract predominantly containing proteins with molecular weights below 30 kDa (see Fig. 3). The predominance of low molecular weight proteins (<30 kDa) in the 80% ethanol extract can be due to ethanol, particularly at higher concentrations, which may preferentially solubilize smaller proteins or peptide fragments over larger ones [37, 44]. This selective solubilization could result from differences in protein denaturation, solubility, and interactions with the solvent environment. Moreover, the presence of low molecular weight proteins in earthworm extracts aligns with findings from Tram et al. [45], who documented protein molecular weights spanning from 14.4 kDa to 116 kDa in earthworms, with only proteins below 30 kDa persisting after hydrolysis. This suggests that earthworms inherently contain a significant proportion of low molecular weight proteins, which may be more readily extracted with certain solvents such as 80% ethanol.

The experiment evaluating the antifungal activity against *C. albicans* revealed varying inhibition zone diameters and MIC for earthworm extracts obtained using different solvents, ranging from 5.3 to 13.3 mm and 75 to 250 mg/mL, respectively (see Table 2). While the antifungal activities of extracts from various earthworm species have been reported [9–13], information on the antifungal activity of extracts from *P. excavatus* in Vietnam remains limited, potentially making this report the first of its kind. Notably, the 80% ethanol extract exhibited the strongest antifungal activity against *C. albicans*, with an inhibition zone diameter of 13.3 mm and an MIC of 75 mg/mL. This potency could be attributed to several factors, including the composition of the extract and its underlying mechanism of action. The observation that the 80% ethanol extract predominantly contains low molecular weight proteins (<30 kDa) suggests a possible correlation between protein content and antifungal activity. These low molecular weight proteins may possess specific bioactive peptides or compounds with potent antifungal properties, enhancing the extract's efficacy against *C. albicans* [46]. Additionally, the mechanism of action of earthworm extracts against *C. albicans* may involve multiple pathways. One potential mechanism is the disruption of fungal cell membranes by bioactive

compounds present in the extract [47]. This disruption can lead to leakage of intracellular contents, loss of membrane integrity, and ultimately, fungal cell death. Certain bioactive peptides or proteins within the extract may interfere with essential fungal processes such as cell wall synthesis, protein synthesis, or DNA replication, thereby inhibiting fungal growth and proliferation [48]. In addition, the presence of secondary metabolites, such as phenolic compounds or alkaloids, in the earthworm extract could contribute to its antifungal activity by exerting oxidative stress on fungal cells or interfering with key cellular pathways [47]. In conclusion, the strong antifungal activity of the 80% ethanol extract against *C. albicans* underscores the potential therapeutic value of earthworm extracts as natural antifungal agents.

The DPPH and ABTS assays were employed to assess the antioxidant activity of earthworm extracts. Results revealed IC_{50} values ranging from 231.3 to 838.7 μg/mL for the DPPH assay and 208.9 to 864.5 μg/mL for the ABTS assay, indicating varied antioxidant potency across extracts obtained using different solvents (see Fig. 5). The antioxidant activity from extracts of other earthworms has been reported [19–21]. However, limited information exists regarding the antioxidant activity of extracts from *P. excavatus*, a species of earthworm found in Vietnam, making this study potentially the first to report on this aspect. Notably, the 80% ethanol extract exhibited the highest antioxidant activity in both the DPPH and ABTS assays, with IC_{50} values of 231.3 and 208.9 μg/mL, respectively. The superior antioxidant activity of the 80% ethanol extract in both DPPH and ABTS assays can be attributed to several factors, primarily the presence of low molecular weight proteins (<30 kDa) within the extract. Low molecular weight proteins are known to possess potent antioxidant properties due to their ability to donate hydrogen atoms or electrons to neutralize free radicals [49–51]. This donation process interrupts the free radical chain reaction, thus mitigating oxidative stress. Additionally, 80% ethanol as a solvent might efficiently extract these low molecular weight proteins along with other bioactive compounds, enhancing the overall antioxidant activity of the extract [37]. This finding highlights the potential therapeutic benefits of earthworm extracts, especially those obtained using 80% ethanol, in combating oxidative stress-related disorders.

CONCLUSIONS

In conclusion, the study demonstrated that the choice of solvent significantly impacts the extraction efficiency and composition of proteinaceous materials from earthworm powder. Aqueous extraction proved to be the most effective method, yielding the highest extraction efficiency and protein content. This was supported by TLC results showing the presence of amino acids and peptides in the extracts. Additionally, FTIR spectroscopy confirmed the presence of characteristic peaks associated with protein structures in all extracts. SDS-PAGE analysis revealed distinct patterns in protein composition, with proteins primarily below 50 kDa and especially proteins below 30 kDa when 80% ethanol extraction was used. Furthermore, the antifungal activity against *C. albicans* varied among extracts, with the 80% ethanol extract exhibiting the highest efficacy. In addition, earthworm powder extracts showed antioxidant activity, with the 80% ethanol extract demonstrating the highest scavenging activity against DPPH and ABTS radicals. These findings underscore the potential of earthworm powder extracts as sources of bioactive compounds with diverse applications in pharmaceutical and cosmetic industries. Further research exploring the bioactive components responsible for these activities and their mechanisms of action is warranted to fully harness the therapeutic potential of earthworm-derived products.

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