

Effect of the coelomic fluid of the earthworm *Aporrectodea tuberculata* in inhibiting the growth of different types of pathogenic bacteria

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Abstract

Earthworms *Aporrectodea tuberculata* that are characterized by their large size, pale pink or gray color, in this study, the coelomic fluid of this worm was used on different types of pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *salmonella typhi*, *Klebsiella pneumoniae* and *E.coli*) using two solvents Petroleum Ether and Acetone. The results showed that the coelomic fluid dissolved with Petroleum Ether was more effective than the coelomic fluid dissolved with acetone when it was treated with *Salmonella* bacteria and its inhibitory activity was estimated at 15.67 mm compared with acetone which recorded an inhibition diameter at the same bacteria 13.67 mm while the AM antibiotic gave an inhibitory activity of 25.00 mm with significant differences between the treatments, there was no significant difference in the inhibitory activity on *Staph* and *Pseudo* bacteria between the two solvents Petroleum Ether and acetone and their effectiveness was estimated by mm, respectively, 14.00 and 13.67 for Petroleum Ether and 12.33, 13.67 for acetone, and the antibiotic did not give any activity against the precursor bacteria. While in *Klebsiella* bacteria, the inhibitory activity of the anti-AM was 13.00 mm very close to that of the dissolved coliform liquid Petroleum Ether, which gave an inhibition rate of 13.33 mm. Its growth inhibition was 12.33 and 11.67 mm, respectively, which was higher in both of them than the AM antibiotic, which recorded an inhibition rate of 10.00 mm.

Keywords: *Aporrectodea tuberculata*, Coelomic fluid, Petroleum Ether, Acetone, Anti-bacterial activity.

1. Introduction

Earthworms are one of the largest groups of invertebrates that have a large evolutionary system, as there are more than (6000) types of earthworms on all continents except Antarctica, as most of the worms live in moist soil rich in organic matter and some of them live in tree waste And the decomposing leaves and on the banks of the rivers (Brown., 2018), earthworms have been used in ancient and modern times as a treatment for many diseases and as an anti-bacterial, in addition to having an anti-coagulant or fibrinolytic activity, which leads to facilitating blood circulation. most of the research on anti-bacterial activity was used through earthworm extracts (Dharmawati et al., 2019).

The earthworm is considered *Aporrectodea tuberculata* is a worm that lives in moist and moderate soils. It is characterized by its large size, pale pink or gray color, ranging between 10 to 20 cm in length, and sometimes looks dark due to the filling of its intestines with soil. It is found in home gardens and agricultural lands, in forests and river banks, and It contains more chemicals than the earthworm *Lumbricus* has a role in bioremediation and preservation of nutritional value in the soil by converting organic waste into a useful by-product known as vermicompost (Hussaini., 2013).

The coelomic fluid of the earthworm is a fluid located

in the space between the body wall and the intestine and is in the form of a milky white fluid. It also contains various molecules that show distinctive biological properties such as antibacterial and anticoagulant substances (C.F.) has the ability to destroy bacterial cells Foreign and contains lysenin protein that inhibits antibacterial activity (Umamaheswarib, 2021). The coelomic fluid in earthworms is secreted through the dorsal pores in the earthworm's skin; This fluid contains many proteases, enzymes and antibacterial proteins (Fiolka et al., 2019; Chen et al., 2017).

Researchers Show (Dash, 2012); Kauschke et al., 2007) that the coelomic fluid has a role in detoxification; It has the ability to destroy bacterial aggression and prevent dehydration. Also, the subsurface fluid contains Lysenin, a protein containing 297 isolated amino acids, which has the ability to permeate into the membrane of bacteria while binding to the lipoprotein such as lock and key (Shogomori and kobayash., 2008). The bacteria are *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus* and typhoid bacteria *Salmonella typhi* are among the most prominent types of bacteria that cause diseases(AL-khafaf., 2021; kaiser et al., 2021), *Staphylococcus aureus* is the most responsible species for superficial skin infections, which accounts for 60% of all bacterial skin infections due to its

virulence factors (Ji et al., 2018), followed by *Pseudomonas aeruginosa* as the second pathogen isolated from cases of skin burns, being It is a dangerous bacterial species that is resistant to many antibiotics (Yongsoon et al., 2018), Excessive use of over-the-counter antibiotics without a prescription has led to bacteria increasing their ability to develop resistance to antibiotics; As multiple antibiotic-resistant strains have arisen (Woodworth et al., 2018), the search for natural antibiotics from animal extracts, including earthworms (Debnath et al., 2016), is being sought. For this reason, the aim of the research was to find natural alternatives that have the ability to inhibit the growth of Different types of pathogenic bacteria.

2. Materials And Methods

Earthworm sample collection

Earthworms were collected from Hammam Al-Alil from the garden of the house and were diagnosed at Al-Mustansiriya University by Dr. Nibras Faleh and it turned out to be *Aporrectodea tuberculata*.

Method of extraction of coelomic fluid

Earthworms are placed in distilled water to get rid of gastrointestinal secretions; As they were quickly washed and dried on filter paper, earthworm samples were taken and stimulated with a 5-volt electric wire (electrical stimulation) to produce coelomic fluid through the dorsal pores of the earthworm epidermis (Hassan et al., 2019).

Then the liquid was collected and placed in a tube at 4°C, and quantities of organic solvents were placed on it, such as acetone and petroleum ether, in order to increase the effectiveness of the materials in the coelomic fluid, Later, the material was evaporated to get rid of the organic solvent and get the raw liquid, and later the liquid was diluted with the solvent to get the concentrations and to perform the inhibitory activity against the bacterial isolates (Tutar and karamen., 2017).

Sensitivity Measurement Method Diffusion by Digging

The inhibitory activity of coelomic fluid was tested on the growth of the bacteria under study. The medium of Muller-Hinton-Akar was inoculated with the bacteria to be tested, the suspension was diluted using saline solution and by comparison with the standard McFarland control tube, which is equivalent to 10 cells/ml, Then 0.1 ml of the bacteria suspension was spread on a Muller-Akar steel plate and using a cotton swab, after waiting ten minutes for the medium containing the cultured bacteria to dry, holes were made in the dishes using Stainless steel borer with a diameter of 6 mm, then we put 0.1 ml of concentrate in each hole coelomic fluid, Then the dishes were incubated at 37 °C for 24 hours and the diameters of inhibition were measured around each hole in mm using a ruler, then the results were recorded and compared with the effect of the antibiotics used under study and mentioned above

(Balouir et al., 2016; Valgas et al., 2007; Annamalia et al., 2009).

3. Statistical Analysis

The data were analyzed according to the simple and factorial experiment system using the complete random design, and the averages were compared using the SAS program, and the mean deviation of the area of inhibition was calculated using Duncan's multiple test at a probability level of 0.01.

4. Results and Discussion

Table (1) showed the difference in the inhibitory activity of coelomic fluid for earthworms *A. tuberculata* on bacteria according to the methods of using the coelomic fluid as well as the type of solvent used, as shown in Table (1) It was found that the coelomic fluid dissolved with Petroleum Ether was more effective than the coelomic fluid dissolved with acetone when it was treated with *Salmonella* bacteria, and its inhibitory activity was estimated at 15.67 mm compared to acetone which recorded a diameter of inhibition at the same bacteria 13.67 mm, while the AM antibiotic gave an inhibitory activity of 25.00 mm with significant differences between treatments, there was no significant difference in the inhibitory activity on *Staph* and *Pseudo* bacteria between the two solvents Petroleum Ether and acetone and their effectiveness was estimated by mm, respectively, 14.00 and 13.67 for Petroleum Ether and 12.33, 13.67. for acetone, Also, the antibiotic did not give any inhibitory activity against the aforementioned bacteria, while in *Klebsiella* bacteria, the inhibitory activity of AM 13.00 mm was very close to that of the dissolved colliary liquid Petroleum Ether, which gave an inhibition rate of 13.33 mm.

As for *E.coli* bacteria, when they were treated with dissolved columbic liquid Petroleum Ether and Acetone, there was a clear significant difference in their growth inhibition, amounting to 12.33 and 11.67 mm, which was higher in both of them than the AM antibiotic, which recorded an inhibition rate of 10.00 mm, while the lowest inhibitory activity was recorded for *Enterococcus* bacteria and it was estimated for the solvents Petroleum Ether and Acetone, respectively 12.00 and 10.33 mm, noting that the antibiotic did not give any inhibition activity *Enterococcus* bacteria, The results of the current study agree with what was indicated by the researcher (Mustafa et al., 2022) that the coelomic fluid possesses many biologically active compounds, enzymes and antigens that play an important role in inhibiting bacteria and also act as anticoagulant factors hence, the development of new invertebrate treatments began to prevent the emergence of resistance in pathogenic bacteria; our study agreed with the researcher Hussain et al., 2021) that the effectiveness of the subsurface fluid against disease-causing bacteria is due to what it contains enzymes or proteins that have a role in inhibiting or limiting

the growth of bacteria. The researchers pointed out (Mickymaray et al., 2020; Papuc et al., 2017) The earthworm possesses biologically active compounds such as Lumbricin-1 peptides, enzymes and antioxidants that play a major role in inhibiting the growth of infectious pathogens. One of the mechanisms that these peptides and

proteins possess is that they act on enzymes in the bacterial cell wall; They work to disrupt the wall by generating free radicals that disrupt the cell membrane, and also inhibit the synthesis of RNA and protein synthesis, and these enzymes interfere with the metabolic pathways of the cell.

Table (1) Inhibitory effect of Aporrectodea tuberculata coelomic fluid on some types of pathogenic bacteria.

A. tuberculata	Ps.aeruginosa	S.aureus	E. coli	S.typhi	K.pneumonia	Entero
Acetone	12.33±0.57 A	13.67±0.57 a	11.67±0.57 b	13.67±0.57 C	9.33±0.57 C	10.33±0.57 B
Petroleum Ether	13.67±0.57 a	14.00±0.00 a	12.33±0.57 a	15.67±0.57 B	13.33±0.57 a	12.00±0.00 A
Antibiotic	0.00± 0.00 b	0.00± 0.00 a	10.00± 0.00 c	25.00± 0.00 A	13.00± 0.00 A	0.00± 0.00 C

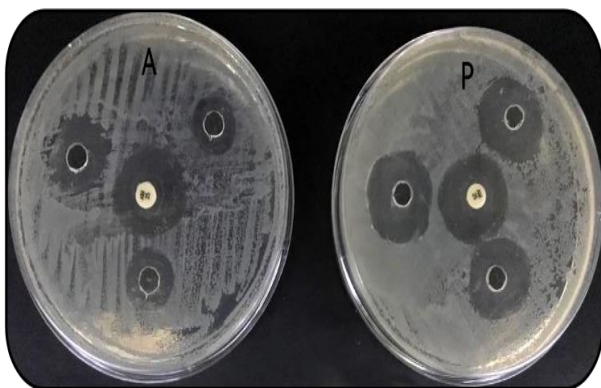


Figure (1): Effect of the earthworm *A. tuberculata* coelomic fluid with petroleum ether(P) and acetone(A) on *S.typhi*.

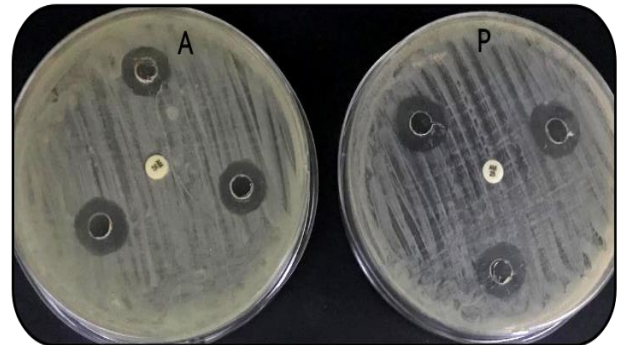


Figure (4): Effect of the earthworm *A. tuberculata* coelomic fluid with petroleum ether(P) and acetone(A) on *S.aureus*

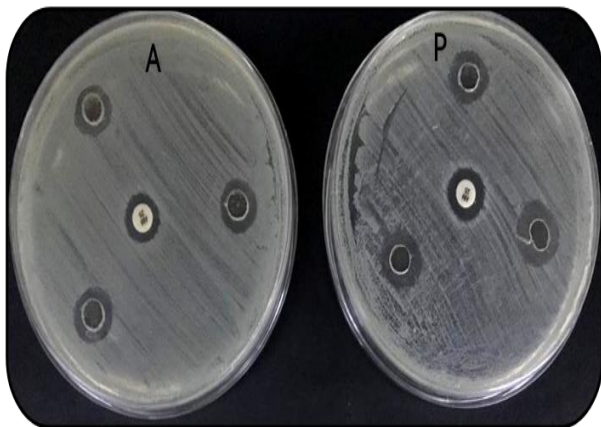


Figure (2): Effect of the earthworm *A. tuberculata* coelomic fluid with petroleum ether(P) and acetone(A) on *K.pneumoniae*

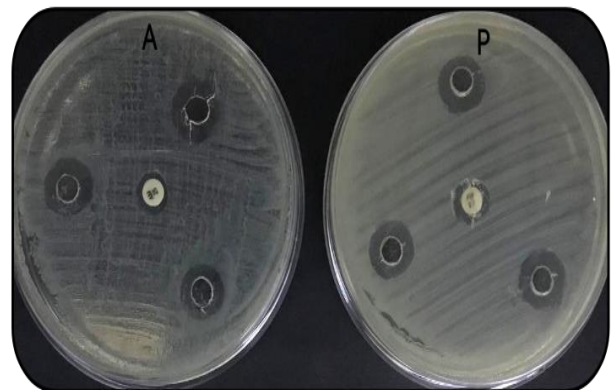


Figure (5): Effect of the earthworm *A. tuberculata* coelomic fluid with petroleum ether(P) and acetone(A) on *E.coli*

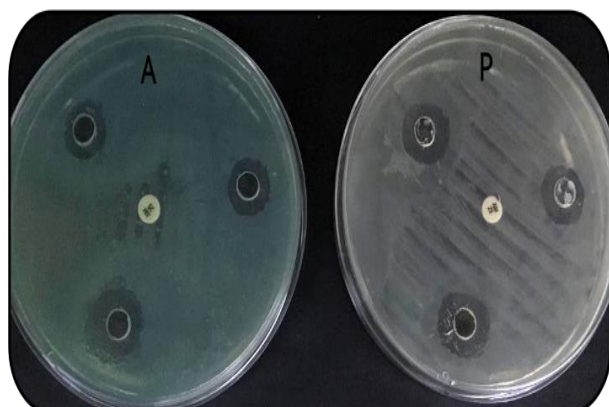


Figure (3): Effect of the earthworm *A. tuberculata* coelomic fluid with petroleum ether(P) and acetone(A) on *Ps. aeruginosa*.

5. Conclusions

The coelomic fluid of earthworms is Aporrectodea. Tuberculata had a significant and clear effect in inhibiting the growth of bacteria, especially when using the solvent Petroleum Ether, higher than the solvent Acetone when it was treated with Salmonella bacteria, and its inhibitory activity was estimated at 15.67 mm compared to acetone, which recorded a diameter of inhibition at the same bacteria 13.67 mm followed by the rest of the bacteria.

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References

- Al-Khafaf, Ab.R.(2021). The effectiveness of some essential plant oil and honey against pathogenic. Bacteria isolated form skin infection. M.Sc. Biology. Coll. Education for pure science Uni. Of mosul.
- Annamalia, N., Manivasagen, P., Balasubramanian, T. and Vajayalakshmi,,S. (2009). Enteronic from *Enterococcus faecium* isolated from mangrove environment. African Journal of Biotechnology, 8(22): 6311-6316.
- Antar, Salem Hammadi Al-Wakaa and Adnan Hussein Ali. (2017) Statistical analysis of agricultural experiments using SAS program. Central Press / University of Diyala, first edition.
- Balour, M.,Sadiki, M. and Ibsouda, S.K.(2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis., 6(2): 71-74.
- Brown K (2018) Earthworm Recorders Handbook Earthworm, Society of Britain 1(4)11 www.earthworm soc.org.uk. ESBengiries. @Gmail Com.
- Chen, P., Day, G., & Czepinski, L. (2017). An Unusual Presentation of a Liver Abscess: *Klebsiella* Liver Abscess Syndrome Disproportionately in East Asian Patients. Proceedings of UCLA Health, 24.
- Dash MC (2012) Charles Darwins plough: Tools for vermiculture technology lk. International publishing House Pvt. Ltd.
- Debnath, Madhusudan, P., Ray choudhury, and Samir Kumar Sil 2016 "Studies on NS⁵ 18 S RDNA based molecular phylogenetic analysis and antibacterial Properties of *Eutyphoeus gammiei*, an earthworm Species
- Dharmawati, I. G. A. A., Mahadewa, T. G. B., & Widyadharna, I. P. E. (2019). Antibacterial activity of *Lumbricus rubellus* earthworm extract against *Porphyromonas gingivalis* as the bacterial cause of periodontitis. *Open Access Macedonian Journal of Medical Sciences*, 7(6), 1032-1036.
- Fiolka, M.J; Czaplewska, p; Macur, K; Buchwald T; Kutkawska, J; Paduch, R. Kaczynski Z: wydrych, J: Urbanik-sypniewka T, Anti candida albicans effect of protein-carbohydrate fraction obtained from the coelomic fluid of earthworm *Dendrobaena Ueneta*, plos one, (2019)4 – e0212869.
- Hassan, Z.N, Ali; BM, Falih. N, (2019). effect of Eearthworm codomic fluidon In hibition Activity Against some Pathogenic Bacteria. Issn: 0975-8542
- Hussain, A. (2013) vermiculture biotechnology and effective tool for economic and environmental Sustainability African Journal of Environmental Science and Technology, 4(2):56-60.
- Hussain, N., Chatterjee, S.K., Maiti, T.K., Goswami, L., Das, S., Deb, U., Bhattcharya, S.S., (2021). Metal induced non-metallothionein protein in earthworm: a new pathway for cadmium detoxification in chloragogenous tissue. J. Hazard. Mater. 401, 123357
- Ji, S. L.; Hyun-Sun, P. and Soyun, C. (2018). Treatment Response in bacterial skin infection *J. Annals of dermatology*, 30(2):186-191
- Kaiser, P.; Wachter, G. and Windberys, M.(2021). Therapy of infected wounds overcoming clinical challenges by advanced drug delivery system. Drug delivery and translation research: 14-35
- Kauschke, E., Mohrig, W., & Cooper, E. L. (2007). Coelomic fluid proteins as basic components of innate immunity in earthworms. *European Journal of Soil Biology*, 43, S110-S115.
- Micky maray. S, Alfaiz.F. A. and Paramasivam A. (2020). "Efficacy and mechanisms of flavonoids against the emerging oppor-tunistic nontuberculous Mycobacteria. Antibiotics no vol.9 no 8, P450.
- Mustafa, R.G. Saiqa, A. Dr. Dominguez, J. Jamil, M. Manzoor, S. Wazir, S. Shaheen, B. Parveen, A. Khan, R. Ali, Sh. Ali, N.M. Jalal, F. Raja, S.A. (2022). Therapeutic Values of Earthworm Species Extract form Azad Kashmir as Anti Coagulant, Antibacterial, and Antioxidant Agents. Canadian Journal of Infectious Disease and Medical Microbiology. Article ID 6949117, 20 Pages
- Papuc. c, Goran G; V, Predescu. C.N Nicolescu Vand Stefan. G. (2017). "Plant enols, as extension and antibacterial agents for shelf-life extension of meat and meat Products, class fication, structures. Sources and action mechanisms comprehensive Reviews in food Science and food safety (2017) Vol. 16 no 6, PP. 1243-1268.
- Shogomori H, Kobayashi T (2008). lysenin a sphingomyelin specific por-forming toxin. *Biochimica et Biophysica Acta (BBA) – General subjects*, 1789(3): 612 – 618.
- Tutar V. And Karamen I, 2017, In vestigation of anti-bacterial properties of mucus and coelomic fluid obtained from *Eisenia fetida* Vol 38-3(2017) 427 – 434.
- Umamaheswari, S., & Murugan, S. (2021). Identification of lysenin protein function in coelomic fluid of *eudrilus eugeniae*. *European Journal of Molecular & Clinical Medicine*, 8(3), 687-696.
- Valgas, C., Souza, S. M. D., Smânia, E. F., and Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*, 38(2): 369-380.
- Wood Worth, K.R.; Walters, M.S; Weiner, L.M; Edwalds, J; Brown A, C; Huang, J.Y.; Mailik, S.; Slayton, R.B; Paul, P; Capers, C.; Kainer, M.A; Wilde, N., Shugart, A; Mahon, G; Kallen, A.J Patel, J; Mcdonald, L.C; Srinivasan, A.; Craig, M. and cardo D.M.(2018) vital signs: containment of novel multidrug Resistant organisms and Resistance mechanisms-united states, 2006-2017. *Weekly*, 67(13): 396-401
- Yongsoon, Ch.; Jin, H. P.; Ji, H. K.; Seung, B. H. and Areum, D. (2018). Clinical predictors of *Pseudomonas aeruginosa* bacteremia in Emergency department *J. Emergency Med. Int.*