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on

Antimicrobial Activity of *Pheretima posthuma* (Kinberg)Extract

by

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Declaration

I hereby declare that this report is an authentic record of work carried out by me under UGC Minor Research Project, MRP(S) – 0231/12-13/KLKA004/UGC-SWRO, Dt. 23 SEPTEMBER 2013 on Antimicrobial Activity of *Pheretima posthuma* (Kinberg)Extract.

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Introduction

During the last half century Ethnobiology has evolved as a valid discipline that can play a prominent role in the advancement of many aspects of scientific, sociological and historical studies. An increasing number of investigations have been done to the vast store of knowledge of plant properties and uses, still intact in native cultures in several parts of the world and comparatively less concentration on ethnozoological studies. The Indian subcontinent represents one of the greatest emporia of ethnobiological wealth and Western Ghats represents the second hotspot in India. In Kerala still many living groups of tribals more or less isolated from the influence of modern world continue to use various domesticated and wild animals and plants for food, drug, game, custom and religious purpose etc. They hunted the animals for bare necessity without tilting the balance of the ecosystem, provided their dietary system with needed nutritive value for the sustenance of life. Animals including birds have so far been proved as quiet useful source of food to the tribals in different areas of the country depending on the availability of specific animals around their habitats. This indicates indirectly the authenticity of usage of such drugs that evolved through ages in the health care system of tribals.

- **Origin of the research problem**

Earthworm plays a major role in the proper functioning of the soil ecosystem. It acts as scavenger and helps in recycling of dead and decayed plant material by feeding on them. Earthworm increases the soil fertility and is often referred to as a farmer's friend. Earthworms have been used as medicine for various remedies by the tribal people. Dried worms are used in healing wounds, chronic boils, piles, sore, chronic cough, diphtheria and jaundice. Oil from worm is used in hemiplegia, paralysis and muscular pain. In the present investigation, various solvent extracts of earthworm *Pheretima posthuma*(Kinberg), were prepared and antimicrobial activity of these extracts were determined by well diffusion method.

- **Interdisciplinary relevance**

1. Ethnozoological importance:

   Documenting medicinal importance of animals to provide an update on indigenous know how.

2. Anthropological importance:

   Animal drugs used by tribals are used for tribal scrutiny.

3. Microbiological importance:

   Assessing the antibacterial and antifungal property of the extract.

4. Pharmacological importance:

   These studies may lead to the formulation of new antimicrobial drug.

Objective

- To assess the antibacterial and antifungal property of *Pheretima posthuma* (Kinberg) extract.

Review of research

- **International status**

Earthworms have been used in medicine for various remedies since 1340 AD [J Stephenson, 1930]. Earthworm has been recognized in oriental medicine as anti- inflammatory, analgesic and antipyretic agent [N Noda; S Tsunefuka; R Tanaka; K Miyahara, 1992].It shows anticancer effect by preventing excess glucose uptake [H Nagasawa; K Sawaki; Y Fuji; M Kobayashi; T Segawa; Suzuki;H Inatomi, 1992]. Microorganisms are known to play a major role in soil characteristics, invertebrates are believed to act as regulators of antimicrobial activity. Earthworm surface excreta were found to have potent antimicrobial activity [AS Oleynik; BA Byzov, 2008]. It is also having anticoagulatory or fibrinolytic activity which results in the facilitation of blood circulation [JD Wang; T Narui; H Kurata; K Takouchi; T Hashimoto; T Okuyana1989]. The earthworm has been suspected to contain proteases which dissolve the fibrin clots or anticoagulants which selectively interfere with the intrinsic pathway of blood coagulation cascade [KG Mann; ME Neseirm; WR Church; S Krishnaswamy,1990; EW Davie; K Fujikawa; W Kisiel,1991; C Leipner; I Tuckova; J Rejnek; J Lagner,1993; YS Kim; YE Kim; HS Byun; CS Chang,1995; J Woo; YK Bank; KH Yu; SR Paik; CS Chang.,1996]. Medicinal properties of earthworm have been described [HS

Bristow,1932; HS Bristow,1938; A Ogata; K Morimoto; H J Mori,1939; R Yegnanarayan; PP Sethi; PA Rajhan; K Pulandiran; SA Ismail,1987]. Antitumor activities of earthworm fibrinolytic enzyme on human hepatoma cells were studied [C Hong,2007].

- **National status**

Earthworms have largely been used internally and externally as powerful aphrodisiacs [SB Vohora; MSY Khan,1978]. Medicinal properties of earthworm have been described [HS Bristow,1932; HS Bristow,1938; A Ogata; K Morimoto; H J Mori,1939; R Yegnanarayan; PP Sethi; PA Rajhan; K Pulandiran; SA Ismail,1987]. Anti-inflammatory activity of earthworm extracts was studied [SA Ismail; K Pulandiran; R Yegnanarayan,1992]. Antimicrobial potency of *Eudrilus eugeniae* extracts on certain plant pathogens were studied [SV Shobha; R Kale,2007]. The anti inflammatory and antipyretic activities of biologically active extract isolated form whole earthworm, *Lampito mauritii* were determined [M Balamurugan; K Parthasarathi; EL Cooper; LS Ranganathan,2008].

- **Significance of study**

Knowledge of medicinal or nutritive quality of all the animal species become the need of the hour. The use of animals for medicinal purposes is a part

of traditional knowledge which is increasingly becoming relevant to discussions on conservation biology, public health policy, sustainable management of natural resources, biological prospection and patents.

Methodology

Collection of earthworms

Fully matured earthworms were collected and the worms were washed in running tap water in order to remove the sand particles from the surface of earthworms. Then after washed earthworms were soaked in N-saline and solution was exchanged after every time so that the gut of earthworms gets thoroughly cleaned.

Preparation of earthworm extracts

The method for preparation of plant extract will be modified [PI Alade, 1993]. About 10 to 20 g of the earthworms were homogenized separately in different solvents used according to decreasing polarity such as that is phosphate buffer (0.2 M, pH 7.0), 95% ethanol and petroleum ether. The homogenized mixtures prepared separately in different solvents were filtered and the filtrates obtained were condensed in water-bath at 350C. The crude extracts obtained were diluted in 10%DMSO for evaluation of antimicrobial activity.

Culture media

The media used for bacterial culture will be Nutrient agar/broth while Sabouraud's Dextrose-Agar(SDA) will be used for fungal cultures.

Inoculum

The bacterial cultures inoculated in Nutrient Agar/broth were incubated at 370C for 18 h. The suspension were checked to provide approximately 105 cfu/ml. Fungal cultures were inoculated in Sabouraud's Dextrose Agar/broth and were incubated at 370 C for 48 h .

Microorganism used

Pure cultures of *Staphylococcus aureus*, *E. coli, Streptococcus pyogens, Aspergillus niger and Candida albicans* will be used.

Determination of antibacterial Activity

The agar well diffusion method [C Perez; M Pauli; P Bazevque, 1990] will be modified. Nutrient agar medium used for bacterial cultures medium will be inoculated with cultures, suspended separately in Nutrient/broth. The total of 8 mm diameter wells were punched into agar and the plates were left free for solidification. The wells were filled with different solvent extract of earthworm

and another plate will be used as positive and negative control. Chloramphenicol (1 mg/ml) will be used as the positive control. Different solvents such as phosphate buffer, 95% ethanol, petroleum ether and 10% DMSO were used as negative controls. The plates were incubated at 370C for 18 h. The antibacterial activity is determined by measuring the diameter of zone of inhibition.

Determination of antifungal activity

Sabouraud's dextrose/agar (SDA) will be used for the growth of fungal culture. The same procedureas that for assaying the antibacterial activity was adopted and fungal cultures were kept for 48 h to determine the diameter of zone of inhibition. Fucanazole (1 mg/ml) will be used as standard positive control and different solvents as mentioned above were used as negative controls.

Determination of MIC

The antibacterial earthworm extracts were then after evaluated to determine MIC values. The serial dilution technique by using N-saline for diluting the earthworm extract was adopted and serially diluted earthworm extract tubes were incubated for 48 h. The minimum dilution of earthworm extract that inhibits the growth of the organism will be taken as MIC.

Results and Discussion

Antimicrobial Activity

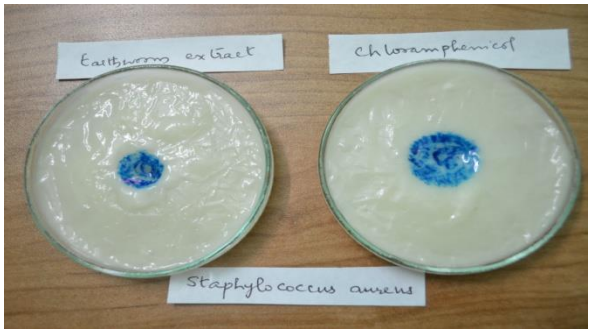
In this study two isolates of gram positive pathogenic bacteria (Staphylococcus aureus and Streptococcus pyogens) and two species of pathogenic fungal strains (Aspergillus niger and Candida albicans) were used to evaluate antimicrobial activity of earth worm extract of Pheretima posthuma.

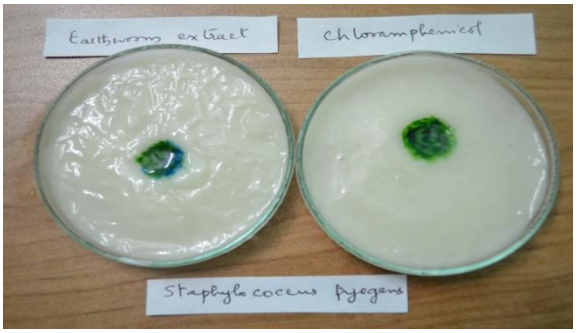
Antimicrobial potency of earthworm extract was evaluated by measurement of zone of inhibition on agar plate after 18 hours of incubation at 37oC by well diffusion method and photographed. The results were tabulated as follows.

Table: 1 Determination of antimicrobial activity by well diffusion method

Sl. No.	Bacterial species	Diameter of zone of inhibition	
		Earth worm Extract 10mg/ml	Chloramphenicol 1mg/ml
1	Staphylococcus aureus	20	31
2	Streptococcus pyogens	21	23

Among the two species tested, Staphylococcus aureus shows maximum antibacterial activity.





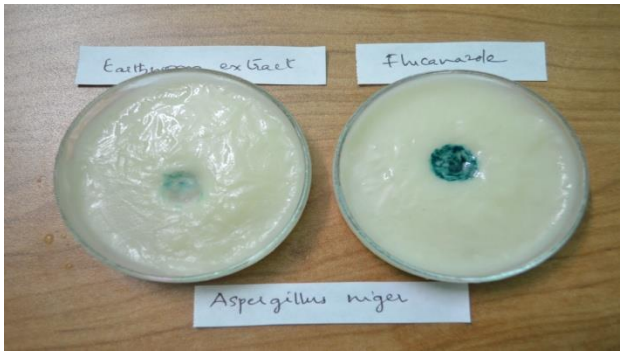
Antifungal Activity

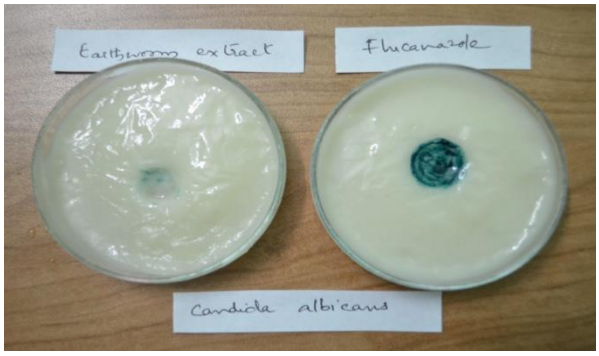
Antifungal activity was evaluated by measurement of zone of inhibition on SDA plate after 48 hours of incubation at 37^{o}C by well diffusion method and photographed. The results are tabulated below.

Table: 2 Determination of antifungal activity by well diffusion method

Sl. No.	Fungal species	Diameter of zone of inhibition	
		Earth worm Extract 10mg/ml	Flucanazole 1mg/ml
1	Aspergillus niger	-	17
2	Candida albicans	-	17.5

Results of antifungal activity of earth worm extract showed that it was not able to inhibit fungal growth.





Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was determined using micro dilution broth method. The extract was diluted to form different concentrations and are evaluated for microbial activity. Bacterial suspension of 0.5ml was added to test tube containing different concentrations of earth worm extract and are incubated. The turbidity was measured after 24 hours. The results were given below.

Table: 3 MIC tested for earth worm extract by optical density method

Bacterial culture	Concentration of Earth worm extract	Optical Density
	Control	25.2
	50µl	14.26

Staphylococcus aureus	100 µl	12.36
	150 µl	7.72
	200 µl	5.78

MIC result indicate that earth worm extract at a dilution of 200 µl inhibit bacterial growth than other dilutions.

References

1. Stephenson J. The Oligochaeta, Oxford University Press, London. 1930.

2. Noda N, Tsunefuka S, Tanaka R and Miyahara K. Effect of an earthworm, *Lumbricus rubellus*. Chem. Pharm. Bull. 1992; 40, 2756.

3. Nagasawa H, Sawaki K, Fuji Y, Kobayashi M, Segawa T, Suzuki R and Inatomi H. Biology of lysenin a protein in the coelomic fluid of earthworms. Anticancer Res. 1991; 1061.

4. Oleynik AS and Byzov BA. Response of bacteria to earthworm surface excreta. Microbiologiya. 2008; 77, 854-862.

5. Wang JD, Narui T, Kurata H, Takouchi K, Hashimoto T and Okuyama T. Fibrinolytic activity of the earthworm extract. Chem. Pharm. Bull.1989; 37, 2236.

6. Mann KG, Neseirm ME, Church WR and Krishnaswamy S. Surface dependent reactions of the vitamin K- dependent enzyme complexes. Blood. 1990; 76, 1.

7. Davie EW, Fujikawa K and Kisiel W. The coagulation cascade: initiation, maintenance and regulation. Biochemistry. 1991; 30, 103-163.

8. Leipner C, Tuckova I, Rejnek J and Lagner J. Serine proteases in coelomic fluid of annelids *Eisenia fetida* and *Lumbricus terrestris*. Comp. Biochem. Physiol.

1993; 105 B, 679.

9. Kim YS, Kim YE, Byun HS and Chang CS. Regulation of NAD+ glycohydrolase activity by ADP ribosylation. J. Biochem. Mol. Bio., 1995; 28, 398.

10. Woo J, Bank YK, Yu KH, Paik SR and Chang CS. Mechanism of Blood coagulation. J. Biochem Mol. Biol. 1996; 29, 500.

11. Bristow HS. Insects and other invertebrates for human consumption in Siam. Transactions of the Entomological Society, 1932; 80,387- 404.

12. Ogata A and Mori HJ. Constituents of the earthworm as an antipyretic agent I. Journal of Pharmacological society of Japan. 1938; 58,859-87.

13. Ogata A, Morimoto K and Mori H J. Constituents of the earthworm as an antipyretic agent II. Ibrid. 1939; 59, 481-494.

14. Carr LGK. Interesting animals, foods, medicines and omens of the eastern Indian with comparison to ancient Europe practice. Journal Washington Academy of Science. 1951; 41,229-235.

15. Yegnanarayan R, Sethi PP, Rajhan PA, Pulandiran K and Ismail SA. Anti-inflammatory activity of total earthworm extracts in rats. Indian Journal of Pharmacology.1987; 19, 221-224.

16. Vohora SB and Khan MSY. Animal origin drugs used in Unani Medical Research-Tughlaquabad, New Delhi, 1978

17. Ismail SA, Pulandiran K and Yegnanarayan R. Anti-inflammatory activity of earthworm extracts. Soil Biol. Biochem. 1992; 24(12), 1253-1254

18. Balamurugan M, Parthasarathi K, Cooper EL and Ranganathan LS. Anti-inflammatory and anti-pyretic activities of biologically active extract isolated from whole earthworm, *Lampito mauritii*. Journal of Ethnopharmacology. 2008.

19. Shobha SV and Kale R. Antimicrobial potency of earthworm, *Eudrilus eugeniae* on certain plant pathogens. Administrator. 2007.

20. Hong C. Earthworm fibrinolytic enzyme and anti-tumor activity on human hepatoma cells, *in vitro* and *in vivo*. Chinese medical Journal.2007.

21. Alade PI; Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana*. J Ethanopharmacol. 1993; 39,171¬174.

22. Perez, C., M. Pauli and P. Bazevque, 1990. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis, 15: 113-115