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## **Antimicrobial Activity of Earthworm Extracts**

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### **ABSTRACT**

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 in medicine for various remedies. In the present investigation, various solvent extracts of an earthworm, *Eudrilus eugeniae* were prepared and antimicrobial activity of these extracts were determined by well diffusion method. It was found that 95% ethanol extract of earthworm was potent antibacterial agent against *Streptococcus pyogens* and antifungal agent against *Candida albicans*. Petroleum ether extract showed maximum potency against *Staphylococcus aureus* in comparison to *Streptococcus pyogens*. Petroleum ether extract was found to possess maximum antifungal activity against *Aspergillus niger* in comparison to *Candida albicans*. Against *E. coli*, ethanol and petroleum ether extract possessed least antibacterial activity. Phosphate buffer extract of earthworm possessed no potency against bacterial and fungal cultures. These studies may lead to the formulation of new antimicrobial drug.

**Keywords:** *Eudrilus eugeniae*, antimicrobial activity, 95% ethanol, petroleum ether, 0.2 M phosphate buffer.

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## INTRODUCTION



Earthworms have been used in medicine for various remedies since 1340 AD [1]. Earthworm has been recognized in oriental medicine as anti-inflammatory, analgesic and antipyretic agent [2]. It shows anticancer effect by preventing excess glucose uptake [3]. 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 [4]. It is also having anticoagulatory or fibrinolytic activity which results in the facilitation of blood circulation [5]. 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 [6-10]. Medicinal properties of earthworm have been described [11-15]. Earthworms have largely been used internally and externally as powerful aphrodisiacs [16]. Anti-inflammatory activity of earthworm extracts was studied [17]. The anti-inflammatory and antipyretic activities of biologically active extract isolated from whole earthworm, *Lampito mauritii* were determined [18]. Antimicrobial potency of *Eudrilus eugeniae* extracts on certain plant pathogens were studied [19]. Antitumor activities of earthworm fibrinolytic enzyme on human hepatoma cells were studied [20]. The species selected for study was *Eudrilus eugeniae*. This species is native of Africa and is having good reproduction and maturation capability. In the present investigation, different solvents were used on the basis of increasing polarity such as petroleum ether, 95% ethanol and 0.2 M (pH, 7.0) phosphate buffer to prepare earthworm extracts in order to assess their antimicrobial activity. This study is a type of new research done ever to use the earthworm extracts against pathogenic microorganisms responsible for causing serious and dreadful diseases.

## EXPERIMENTAL SECTION

All the chemicals and reagents used were from C.D.H and Ranchem. Glass wares used were from Borosil. The media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India.

### Collection of earthworms

Fully matured earthworms were collected from Jay Bharat Nursery, Ranipokhri, Rishikesh (U.K.) India, 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 was modified [21]. 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 35°C. The crude extracts obtained were diluted in 10% DMSO for evaluation of antimicrobial activity.

### **Culture media**

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

### **Inoculum**

The bacterial cultures inoculated in Nutrient Agar/broth were incubated at 37°C for 18 h. The suspension were checked to provide approximately 10<sup>5</sup> cfu/ml. Fungal cultures were inoculated in Sabouraud's Dextrose Agar/broth and were incubated at 37°C for 48 h.

### **Microorganism used**

Pure cultures of *Staphylococcus aureus*, *E. coli*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans* were obtained from Dept. of Microbiology, Sai Institute of Paramedical and Allied Sciences, Dehradun (U.K), India.

### **Determination of antibacterial Activity**

The agar well diffusion method [22] was modified. Nutrient agar medium used for bacterial cultures medium was 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 was used as positive and negative control. Chloramphenicol (1 mg/ml) was 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 37°C for 18 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition.

### **Determination of antifungal activity**

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

### **Determination of MIC and MBC**

The antibacterial and antifungal earthworm extracts were then after evaluated to determine MIC and MBC 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 the earthworm extract that kills the bacterial and fungal growth was taken as MLC (Minimum lethal count) while the minimum dilution of earthworm extract that inhibits the growth of the organism was taken as MIC.

## RESULTS AND DISCUSSION

**Determination of antibacterial activity**

The antibacterial activity was determined by measuring the diameter of zone of inhibition recorded. Ethanolic extract of earthworm possessed maximum antibacterial activity in comparison to petroleum ether extract against *Streptococcus pyogenes*. The diameter of zone of inhibition observed was 19 mm. In comparison to *S. pyogenes*, less diameter of zone of inhibition was observed against *Staphylococcus aureus* (18 mm) followed by *E. coli* (15 mm). Phosphate buffer extract of earthworm does not possess any antimicrobial activity in comparison to ethanolic and petroleum ether extracts. Solvents 95% ethanol, petroleum ether and phosphate buffer showed no zone of inhibition against the respective bacteria, thus were treated as negative controls. Chloramphenicol (1mg/ml) was used as the positive control and possessed maximum diameter of zone of inhibition against *S. aureus* (53mm) followed by *S. pyogenes* (50mm) and *E. coli* (37 mm). (Table 1, Fig.1)

**Table – 1 : Determination of Antibacterial activity by well-diffusion method**

Microorganisms	Diameter of zone of inhibition (mm)		
	Ethanolic extract	Petroleum ether extract	Phosphate buffer extract
<i>Staphylococcus aureus</i>	18	15	NA
<i>Streptococcus pyogenes</i>	19	13	NA
<i>E. coli</i>	15	10	NA

Microorganisms	Diameter of zone of inhibition (mm)			
	Chloramphenicol(1mg/ml)	E	PE	PB
<i>Staphylococcus aureus</i>	53	NA	NA	NA
<i>Streptococcus pyogenes</i>	50	NA	NA	NA
<i>E. coli</i>	15	NA	NA	NA

*E*, Ethanol; *PE*, Petroleum ether; *PB*, Phosphate buffer; *NA*, No Activity





**Fig.1 Determination of Antibacterial activity by well diffusion method**

#### **Determination of Minimum inhibitory Concentration (MIC) and Minimum bactericidal Cone (MBC) of Earthworm Extract**

The MIC and MBC values of earthworm extract were determined according to the serial dilution technique (Table 2).

**Table – 2 : Determination of MIC and MBC of ethanolic extract of earthworm**

Microorganism	MIC (mg/g)	MBC (mg/g)
<i>Staphylococcus aureus</i>	0.01	0.1
<i>Streptococcus pyogenes</i>	0.01	0.1
<i>E. coli</i>	0.001	0.01

#### **Determination of antifungal activity**

Well diffusion method was adopted for determination of antifungal activity. Ethanolic extract of earthworm possessed maximum antifungal activity against *Candida albicans* in comparison to *Aspergillus niger*. The diameter of zone of inhibition observed was 15 mm followed by *Aspergillus niger* (12 mm). The petroleum ether extract of earthworm possessed minimum antifungal activity in comparison to ethanolic extract. Phosphate buffer extract showed no antifungal activity. Fluconazole (1mg/ml) was used as the positive control and possessed maximum antifungal activity in comparison to earthworm extract (Table 3, Fig. 3).

Table – 3: Determination of Antifungal activity of by well diffusion method

Microorganisms	Diameter of zone of inhibition (mm)		
	Ethanolic extract	Petroleum ether extract	Phosphate buffer extract
<i>Aspergillus niger</i>	12	08	NA
<i>Candida albicans</i>	15	07	NA

  

Diameter of zone of inhibition (mm)				
Microorganisms	Fucanazole(1mg/ml)	E	PE	PB
<i>Aspergillus niger</i>	22	NA	NA	NA
<i>Candida albicans</i>	25	NA	NA	NA

E, Ethanol; PE, Petroleum ether; PB, Phosphate buffer; NA, No Activity



Fig. 3 Determination of Antifungal activity

In the present study it has been determined that 95% ethanolic extract of earthworm acts as the potent antibacterial agent against *Streptococcus pyogens* and antifungal agent against *Candida albicans*. Petroleum ether extract showed maximum potency against *Staphylococcus aureus* in comparison to *Streptococcus pyogens*. Petroleum ether extract was found to possess antifungal activity against *Aspergillus niger* in comparison to *Candida albicans*. Against *E. coli*, ethanolic and petroleum ether extract possessed least antibacterial activity. Phosphate buffer extract of earthworm possessed no potency against any of the bacterial and fungal cultures. This study may thus lead to formulation of new natural antimicrobial agent and thus may found beneficial in future prospects for mankind. Thus solvent extracts of earthworm can be used against serious and dreadful pathogenic microorganisms responsible for causing serious pathogenic disorders.

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